BRACHIAL ARTERY RESPONSE TO REACTIVE HYPEREMIA:
Describing High-Flow-Mediated Constriction in Healthy Children, Adolescents and Adults, and the Intra- and Inter-day Reproducibility of High-Flow-Mediated Constriction Response in Adults

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Dedication

This dissertation is dedicated to “Grandpa”, Dr. Joseph Gibilisco. The science projects we did together when I was in elementary school started my love of science and discovery.

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CHAPTER 1. INTRODUCTION

Endothelial dysfunction has been shown to be an important precursor in the atherosclerotic process (Bonetti, Lerman & Lerman, 2003; Ras et al., 2013; Ross, 1993, Ross, 1999; Vita & Keaney Jr, 2002). An association of endothelial dysfunction with increasing age (Celermajer et al., 1994), gender (Juonala et al., 2008; Kapuku et al., 2004), cigarette smoking (Lekakis et al., 1998), and obesity (Tounian et al., 2001; Williams et al., 2005) has been reported.

A widely used method of assessing endothelial function is flow-mediated dilation (FMD) (Celermajer et al., 1992; Corretti et al., 2002; Ras et al., 2013). This method of vascular health assessment utilizes ultrasound imaging and has historically focused on the degree of vessel dilation in response to reactive hyperemia to ascertain endothelial function (Corretti et al., 2002; Flammer et al., 2012; Harris et al., 2010; Thijssen et al., 2011). The response of differing vascular beds, such as radial (Jiang et al., 2011), brachial (Corretti et al., 2002) and femoral (Kooijman et al., 2008) arteries, have been investigated with FMD assessment. All three vascular beds display nitric oxide-mediated responses (Doshi et al., 2001; Joannides et al., 1995 Kooijman et al., 2008), but the brachial artery's ideal location (easily accessible) and vessel diameter makes it the preferred site of assessment (Charakida et al., 2010).

A strong correlation between endothelial function in coronary and brachial arteries has been reported (Takase et al., 1998) which allows brachial FMD to be a surrogate measure of coronary function. Also, many studies have shown impairment of FMD in response to a range of cardiovascular risk factors (Maruhashi et al., 2013; Ras et al., 2013; Yang et al., 2014) and FMD has been inversely associated with future CVD

events (Brevetti et al., 2003; Katz et al., 2005; Muiesan et al., 2008; Ras et al., 2013; Rossi et al., 2008). However, specifically in the brachial and radial arteries, a biphasic response, where vessel constriction occurs prior to dilation, has been observed during reactive hyperemia (Dobrosielski et al., 2006; Jiang et al., 2011).

The constriction phase during reactive hyperemia has been largely overlooked in FMD research. Recent research has examined a constriction effect of the radial vessel during distal occlusion termed low-flow-mediated constriction (L-FMC), and suggests its usefulness as an alternative measure of cardiovascular response to FMD (Dawson et al., 2012; Gori et al., 2008; Gori et al., 2010; Humphreys et al., 2014; Harrison et al., 2011; Weissgerber et al., 2010). In contrast, little is known concerning a constriction of the vessel following distal occlusion (Dobrosielski et al., 2006; Jiang et al., 2011), termed high-flow-mediated constriction (H-FMC). Therefore, the purpose of the following dissertation was to perform a cross-sectional examination of children, adolescents and adults to quantify the frequency and magnitude of H-FMC, determine potential influences of sex, age, body composition, cardiovascular and metabolic factors on H-FMC, and report the reproducibility of an H-FMC in young adults. The findings of the present dissertation will lend additional insight to the existing body of research surrounding FMD assessment and quantify the initial brachial artery response to reactive hyperemia in humans.

The specific study aims arising from this dissertation are as follows:

1. Determine the reproducibility of an H-FMC in healthy young adults.

a. *Hypothesis 1:* H-FMC in young adults will be highly reproducible and have

similar intra- and inter-day reproducibility as FMD.

2. Describe the frequency of H-FMC during reactive hyperemia in normal weight, overweight and obese children and adolescents. Also, investigate differences in FMD, hemodynamic and anthropometric measures between those subjects who experience this phenomenon and those who do not.

a. *Hypothesis 1:* H-FMC will be associated with lower peak FMD. If H-FMC is associated with lower peak FMD, and a decreased FMD is an indicator of endothelial dysfunction, then H-FMC may be considered detrimental. Normal weight children and adolescents will not display H-FMC. A majority of obese and overweight children and adolescents will present H-FMC.

3. Describe the frequency and magnitude of an H-FMC during reactive hyperemia in healthy adult males and females. Additionally, examine the relationship of H-FMC to FMD, biomarkers of cardiovascular and metabolic risk as well as measures of body composition.

a. *Hypothesis 1:* H-FMC will be associated with lower peak FMD and will be significantly correlated with body composition as well as known cardiovascular and metabolic risk factors.

The second chapter of this dissertation will provide an extensive review of the existing literature on arterial vasoregulation. Specific mechanisms of vascular function will be described. FMD, L-FMC and H-FMC methodologies, along with commonly used calculations to quantify vascular function will be examined. Additionally, vascular

dysfunction associated with atherosclerosis, inflammation, body composition and hypercholesterolemia will be discussed.

The third chapter will evaluate whether an H-FMC response is reproducible within and between days in a population of young adults. Flow-mediated dilation has been shown to have very good reproducibility within and between days in an adult population. For H-FMC to be an ancillary measure of endothelial function and vascular health, it would be essential for an H-FMC to be highly reproducible in an individual. The reproducibility of an H-FMC in adults has never been studied, however, understanding the reproducibility could improve the methods of vascular health assessment.

The fourth chapter will examine the presence and magnitude of an H-FMC prior to FMD in children and adolescents across a wide range of body mass indexes and percent body fat. No study has investigated the presence and magnitude of an H-FMC in children and adolescents. Obesity has been associated with negative influences on vascular physiology and there have been an increasing number of obese children and adolescents in the past half-century. The potential correlations of H-FMC to hemodynamic and body composition measures in children and adolescents will be evaluated.

The frequency and magnitude of H-FMC of the brachial artery in adults and the potential relationship of H-FMC with body composition, cardiovascular and metabolic risk factors will be examined in chapter five. To our knowledge, only one study has investigated H-FMC in the brachial artery in adult males, and to date, no studies have

investigated an H-FMC in adult males and females. Also, the inclusion of precise body composition, cardiovascular and metabolic measures has not been examined in association with H-FMC.

Finally, the sixth chapter will summarize each study and the significant observations will be reviewed. Future research involving H-FMC will be proposed.

CHAPTER 2. REVIEW OF LITERATURE

Introduction

With such an overwhelming presence of CVD in the United States and the world (Kochanek et al., 2011; Mendis et al., 2011), there has been great interest in the mechanisms involved in the progression of CVD. Also, the advancement of early detection methods concerning CVD development has intensified in order to prevent future cardiovascular events. A substantial amount of research has specifically investigated the role of the endothelium in the progression of CVD (Bonetti, Lerman & Lerman, 2003; Ras et al., 2013; Ross, 1993; Ross, 1999; Vita & Keaney Jr, 2002).

Vessel function is greatly affected by endothelial-derived substances, which are involved in a host of both pathological and physiological processes (Harris et al., 2010; Rubanyi, 1993; Vane, Anggard & Botting, 1990). Factors such as inflammation and body composition (Bray, 2004) can affect the release of endothelial-derived substances. When the homeostatic balance of endothelial-derived substances (i.e. relaxing and contracting factors) is disrupted, the vasculature is prone to leukocyte adhesion (Granger & Senchenkova, 2010), mitogenesis, thrombosis (Rubanyi, 1993), vascular inflammation, platelet activation (Landray et al., 2004), pro-oxidation, impaired coagulation (Goldberg, 2009), vasoconstriction, and atherosclerosis (Davignon & Ganz, 2004; Grover-Paez & Zavalza-Gomez, 2009). Thus, the complexity of the endothelium and endothelial-derived substances makes it is difficult to define endothelial dysfunction. However, endothelial dysfunction generally refers to vascular reactivity, but can include an inflammatory and thrombotic component.

Various techniques have been developed to evaluate endothelial function

(Brevetti, Schiano & Chiariello, 2008). However, a widely used, non-invasive method of assessing endothelial function is FMD (Corretti et al., 2002; Harris et al., 2010; Thijssen et al., 2011;). L-FMC has been a proposed method complementing FMD for assessing endothelial function (Gori et al., 2008; Gori et al., 2010; Gori, Parker & Munzel, 2011). Interestingly, minimal attention has been given to the H-FMC response of the vasculature to reactive hyperemia (Dobrosielski et al., 2006; Jiang et al., 2011). Recently, the statistical analysis and reporting of FMD and L-FMC as a percent change from a baseline diameter has been challenged (Atkinson & Batterham, 2015; Atkinson & Batterham, 2013). Allometric scaling has been suggested as a way to appropriately quantify a biological change (Atkinson & Batterham, 2015).

The following literature review will first describe the mechanisms of vascular function, and then briefly discuss the relationship of endothelial dysfunction and specific CVD risk factors. Finally, the history and methodologies of FMD, L-FMC and H-FMC will be discussed along with the current debate of allometric scaling.

Mechanisms of Vascular Function

The flow of blood through the peripheral arteries is controlled by a number of components, such as pressure, viscosity and vessel diameter (Kenney, Wilmore & Costill, 2015). The changing of a vessel diameter through constriction or relaxation of the smooth muscle is essential for appropriate distribution of nutrients and removal of waste. Local metabolites sensed by chemoreceptors, pressure changes sensed by baroreceptors, and endothelial-derived substances all have the ability to affect vessel diameter (Kenney,

Wilmore & Costill, 2015). However, endothelial-derived substances will be the focus of the review, with a brief discussion of the myogenic response affecting vessel diameter.

Myogenic Response

An important factor in blood flow regulation is the myogenic response of the vasculature. The definition of a myogenic response is the inherent ability of arterial vessels to response to changes in transmural pressure via diameter modification (Lott, Herr & Sinoway, 2002; Schubert & Mulvany, 1999). Specifically, an increase in pressure across the arterial wall produces smooth muscle contraction while decreased pressure produces relaxation (Kenney, Wilmore & Costill, 2015). This mechanism is independent of endothelial (Ekelund et al., 1992; Falcone, Davis & Meininger, 1991) and neural effects (Folkow, 1949; Folkow, 1952). In limited human research, changes in transmural pressure produced changes in limb flow dynamics at rest and following muscle contraction without significant changes in diameter of the brachial artery (Lott, Herr & Sinoway, 2002; Shoemaker, Pozeg & Hughson, 1996). However, in the radial artery, initial changes in vessel diameter following occlusion have been attributed to changes in transmural pressure (Jiang et al., 2011).

Endothelial-Derived Substances

Vasodilation

The endothelium is a major contributor to vasoregulation, as it produces both vasoconstrictors and vasodilators. The vasodilating factors include: nitric oxide (NO),

prostacyclin, bradykinin, and endothelial-derived hyperpolarizing factor (EDHF) (Vogel, 2001). Of the known vasodilators, NO is the primary factor (Furchgott & Zawadzki, 1980; Palmer, Ferrige & Moncada, 1987). Furchgott and Zawadzki first discovered nitric oxide in 1980, using rabbit atrial vasculature and administration of acetylcholine (ACh) *in vitro* to produce smooth muscle relaxation. Initially termed as an endothelial-derived releasing factor (EDRF), this groundbreaking study demonstrated that for ACh to produce a vasodilatory response *in vitro*, the presence of endothelium was required (Furchgott & Zawadzki, 1980). Subsequently, EDRF and NO were discovered to be synonymous (Moncada, Radomski & Palmer, 1988; Palmer, Ferrige & Moncada, 1987). The synthesis of NO involves nitrogen oxidation from L-arginine to produce L-citrulline and NO (Palmer, Ashton & Moncada, 1988) and this process is catalyzed by endothelial nitric oxide synthase (eNOS) (Forstermann & Munzel, 2006). There are two endothelial forms of NOS: constitutive NOS (cNOS) and inducible NOS (iNOS) (Klabunde, 2011). Nitric oxide is continually being produced by cNOS under normal, basal conditions, while iNOS activity is stimulated during inflammation (Klabunde, 2011). Once produced, NO diffuses to the vascular smooth muscle to activate vasodilation through increases in cyclic guanosine monophosphate (cGMP) (Deanfield, Halcox & Rabelink, 2007; Vogel, 2001). The presence of shear stress on the vessel wall is a vital activator of eNOS. At rest, low levels of NO are constantly released from the endothelium (Vallance, Collier & Moncada, 1989). However, when increased blood flow occurs, the elevated shear stress on the endothelium stimulates elevated NO formation through increased calcium release, thereby causing rapid smooth muscle vasodilation (Klabunde, 2011).

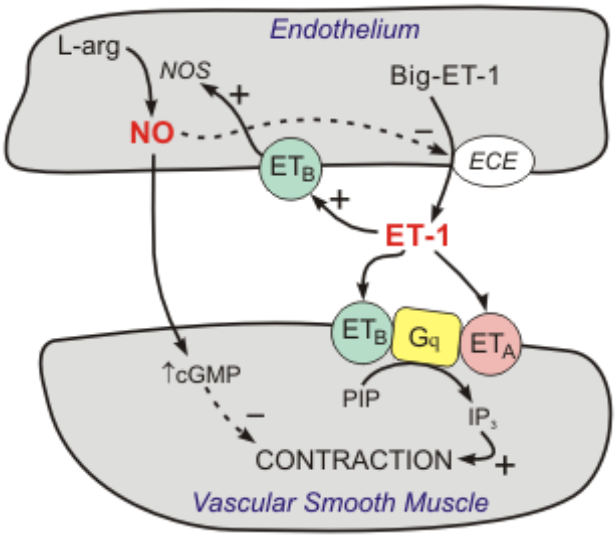
Prostacyclin, a principle metabolite of arachidonic acid (Moncada et al., 1976; Moncada & Vane, 1979), acts independently of NO and has limited involvement in the vasodilator response (Deanfield, Halcox & Rabelink, 2007). Bradykinin also has a secondary effect in vasodilation by stimulating specific endothelial β_2 receptors, which cause the release of prostacyclin (Barrow et al., 1986), NO (O'Kane, et al., 1994) and endothelial-derived hyperpolarizing factor (EDHF) (Hornig, Kohler & Drexler, 1997; Mombouli et al., 1992). Defined as a non-characterized endothelial factor, EDHF has been correlated to an intracellular concentration of Ca^{2+} and membrane hyperpolarization in endothelial cells and vascular smooth muscle cells. When the membrane of vascular smooth muscle is hyperpolarized, a decrease in Ca^{2+} occurs which leads to relaxation (Johns et al., 1988; Luckhoff et al., 1988).

Vasoconstriction

Known endothelium-derived contracting factors are endothelin, thromboxane and angiotensin II (Vogel, 2001). Of these contractile factors, endothelin, specifically endothelin-1 (ET-1), is the most potent (Van Guilder et al., 2007). ET-1 is a 21-amino acid peptide (Yanagisawa et al., 1988) that is produced through the conversion of a 39-amino acid precursor by an endothelin converting enzyme (ECE) (Klabunde, 2011). ET-1 is released from the endothelium and binds to adjacent smooth muscle membrane receptors. The binding of ET-1 causes an increase in Ca^{2+} release by way of a phospholipase C-inositol triphosphate pathway (Luscher & Barton, 2000; Miyauchi & Masaki, 1999; Van Guilder et al., 2007) and produces smooth muscle contraction. Of

interest, ET-1 can stimulate NO production when binding to endothelin receptors found on the endothelium (Figure 1) (Klabunde, 2011). Endogenous production of ET-1 contributes to the maintenance of basal vascular tone (Haynes & Webb, 1994). However, increased ET-1 production can instigate oxidant stress leading to endothelial dysfunction and vascular remodeling (Amiri et al., 2004).

Figure 1. Endothelin Receptors and Interactions with Nitric Oxide



Adapted from Cardiovascular Physiology Concepts, 2nd Edition

Angiotensin II has a similar effect, albeit not as potent, as ET-1 by causing a cascade effect via G protein signaling on the vascular smooth muscle. The G-protein signaling pathway promotes Ca^{2+} release and subsequent constriction (Mehta & Griendling, 2007). Thromboxane is similar to prostacyclin, in that it is formed from arachidonic acid. However, thromboxane has been shown to have a contractile effect on

vascular smooth muscle (Dusting, Moncada & Vane, 1978). Many of the endothelium-derived contracting factors have an inhibitory effect on endothelium-derived relaxing factors, and vice versa.

There is a synergistic approach to maintain vascular tone. Of the previously discussed endothelial-derived vasoactive substances, the primary factors in vasomotor control are NO and ET-1. At rest and in the absence of sympathetic nerve activity, myogenic contraction of smooth muscle in response to pressure (Davis & Hill, 1999) and NO release from the endothelium (Davies, 1995) maintain vascular tone (Thomas & Segal, 2004).

Endothelial Dysfunction and Risk Factors

The endothelium performs a multitude of actions through the production of many vasoactive factors. When the homeostatic balance of endothelial-derived relaxing and contracting factors is disrupted, the vasculature is prone to leukocyte adhesion (Granger & Senchenkova, 2010), thrombosis, vascular inflammation, platelet activation (Landray et al., 2004), pro-oxidation, impaired coagulation (Goldberg, 2009), vasoconstriction, and atherosclerosis (Davignon & Ganz, 2004; Grover-Paez & Zavalza-Gomez, 2009). Many cardiovascular and metabolic disorders and risk factors appear to be in conjunction with endothelial dysfunction (Forstermann & Munzel, 2009). The association between endothelial dysfunction with inflammation, atherosclerosis, obesity and hypercholesterolemia will be discussed.

Inflammation

It has been hypothesized that endothelial dysfunction could be linked to increased inflammatory responses (Grover-Paez & Zavalza-Gomez, 2009). A number of observational studies have described an association of inflammation and CVD risk (Ross, 1999; Stenvinkel, 2001). Specifically, endothelial exposure to pro-inflammatory cytokines can increase adhesion molecules on the endothelial cells (Alexander, 1995) and impair endothelial-dependent dilation (Bhagat & Vallance, 1997). These cytokines could be present from obesity (Anderson, Rahmutula & Gardner, 2004; Wassman et al., 2004), diabetes, or smoking (Barbieri et al., 2011; Jefferis et al., 2010; Wannamethee et al., 2005) and can lead to atherosclerosis. A common serum measure of inflammation, C-reactive protein (CRP), has been inversely correlated with forearm blood flow response to acetylcholine (Fichtlscherer et al., 2000). Interestingly, when CRP levels were decreasing over a 3-month period, endothelial-dependent forearm blood flow-responses normalized (Fichtlscherer et al., 2000). It has also recently been observed that CRP levels are inversely related to basal endothelial nitric oxide synthesis (Cleland et al., 2000). This response is not limited to CHD patients (Fichtlscherer et al., 2000; Sinisalo et al., 2000), but also occurs in healthy adults (Cleland et al. 2000).

Research concerning inflammation and CVD risk is not limited to observational studies. On the contrary, experimental studies have yielded valuable information concerning an inflammation and endothelial dysfunction relationship. One study particularly elicited a temporary, mild inflammatory response to a Salmonella typhi vaccine to produce profound endothelial dysfunction of the arterial vasculature in 12

healthy adults (Hingorani et al. 2000). These findings suggest that an important component in the relationship between low-grade chronic inflammation and CVD is endothelial dysfunction (Grover-Paez & Zavalza-Gomez, 2009).

Atherosclerosis

The combination of inflammation and endothelial dysfunction has been considered a significant precursor to atherosclerosis (Grover-Paez & Zavalza-Gomez, 2009). Laminar shear stress generates a mean positive shear stress on the endothelium and is essential for normal endothelial activation and reorientation (Dewey et al., 1981; Flaherty et al., 1972; Traub & Berk, 1998). However, when low mean shear stress and oscillatory shear stress (i.e. periodic flow reversal with time-averaged shear stress approaching zero) occur, endothelial cells do not reorient (Davies et al., 1986) and could encounter high shear gradients (Davies, 1995). Oscillatory and low mean shear stress conditions are responsible for atherosclerotic lesions, plaque formation and plaque vulnerability (Asakura & Karino, 1990; Ku et al., 1985; Traub & Berk, 1998) and can increase ET-1 expression along with decreasing eNOS expression (Grover-Paez & Zavalza-Gomez, 2009). The increase in ET-1 may lead to vascular inflammation, as ET-1 is associated with pro-inflammatory cytokines like tumor necrosis factor α (TNF-α) and interferon-γ (IFN-γ) (Klemm et al., 1995; Saleh et al., 1997). Endothelin-1 also promotes excessive oxidative stress (Dong et al., 2005), induces adhesion molecules on endothelial cells (Zouki et al., 1999), and mediates nuclear factor-kB (NF-kB) in monocytes (Grover-Paez & Zavalza-Gomez, 2009).

A normal functioning endothelium does not support binding of white blood cells (Grover-Paez & Zavalza-Gomez, 2009). When NO is being regularly produced in response to laminar shear stress, the anti-inflammatory properties of NO can limit the expression of vascular cell adhesion molecule-1 (VCAM-1) on the endothelium. However, augmented production of leukocyte adhesion molecules is experienced during disturbed blood flow. VCAM-1 tends to bind to leukocytes found in atheroma, or the fatty material of plaque (Cheng et al., 2006; Cybulsky et al., 2001; Grover-Paez & Zavalza-Gomez, 2009; Ishizuka et al., 1999). These endothelial-bound leukocytes will enter the intima and propagate a localized inflammatory response thought to precede atherosclerosis. The leukocytes present receptors for low-density lipoproteins to oxidize and lead to foam cell formation (Aviram, 1999). With advanced foam cell formation and enhanced pro-inflammatory cytokine presence (TNF-α and IFN-γ), the release of a variety of peptide growth factors can promote the formation of a dense extracellular matrix that is observed in advanced atherosclerosis (Grover-Paez & Zavalza-Gomez, 2009). This plaque may be localized along one segment of the arterial vasculature or circumferentially (Verstraete, 1990). A hard, calcified plaque would be predominantly fibrotic, while a soft plaque would mainly contain cholesterol-esters and foam cells (Verstraete, 1990). The soft plaque generally has a thin fibrous cap that is more prone to rupture from increased shear stress. Once a fibrous cap has been dislodged, it may develop into a thrombus causing an embolism. Depending on the location of the embolism, stroke (Arenillas, 2011), myocardial ischemia and infraction, or death could occur (Verstraete, 1990).

Obesity

Once considered merely an effecter of healthiness and beauty, obesity is now classified in the same chronic disease stratum as hypertension and atherosclerosis (Avogaro & de Kreutzenberg, 2005; Bray, 2004) and has been correlated to cardiovascular risk factors (Avogaro & de Kreutzenberg, 2005; Stern, 1995). Recently, the regional distribution of fat has been of interest in morbidity and mortality rates. Excessive accumulation of fat in the abdominal region, i.e. central obesity, has been reported as being a better predictor of morbidity and an independent risk factor for coronary heart disease (CHD) (Avogaro & de Kreutzenberg, 2005; Clark et al., 1994; Hodgson et al., 1994; Kaplan, 1989; Kortelainen, 1996). Also, the relationship between CHD and obesity is not limited to older adults, but has been reported in children and adolescents (Must et al., 1992; Nieto, Szklo & Comstock, 1992).

Since adipose tissue has secretory properties, it has the ability to significantly affect the endothelial function. Some of the prominent adipokines, or proteins secreted by adipocytes, are leptin, resistin, interleukin (IL) 6, and TNFα (Avogaro & de Kreutzenberg, 2005). Leptin is the main protein that is released by adipocytes and it has a significant spectrum of effects on vascular homeostasis (Correia & Haynes, 2004). Considering endothelial function, leptin has been shown to cause oxidative stress *in vitro* by increasing reactive oxygen species (ROS) generation (Bouloumie et al., 1999). Increased pro-inflammatory cytokine secretions, such as TNFα and IL-6, can also be stimulated by leptin and affect endothelial function (Bullo et al., 2003; Chu, Chang &

Shieh, 2003). Another adipokine, resistin, has been correlated with the development of insulin resistance, obesity (Steppan et al., 2001), and modified endothelial function (Verma et al., 2003). An increase in ET-1 was observed in endothelial cells that were incubated with human recombinant resistin without a significant change in NO production (Verma et al., 2003). Also, VCAM-1 expression was elevated in resistin-treated endothelial cells, suggesting that resistin promotes ET-1 release from endothelial cells and an upregulation of adhesion molecules (Avogaro & de Kreutzenberg, 2005).

Adipose tissue, specifically visceral adiposity, seems to be a prime regulator of inflammation through direct secretion of pro-inflammatory cytokines. When pro-inflammatory cytokines are released from adipose tissue, they can influence both glucose metabolism and endothelial function (Yudkin, 2003). Adipose tissue is able to stimulate nuclear transcription factor-kappa B (NFκB) by releasing TNFα. NFκB is essential in an inflammatory and apoptotic response by regulating growth factor expression, pro-inflammatory cytokines and adhesion molecules (Mercurio & Manning, 1999). Studies have demonstrated that TNFα released from adipose tissue increases endothelial permeability (Dodd-O et al., 2004) and inhibits eNOS gene expression enough to account for the displayed endothelial dysfunction (Anderson, Rahmutula & Gardner, 2004). Thus, TNFα generates oxidative stress that can lead to endothelial dysfunction and atherosclerosis.

IL-6 also has negative effects on the endothelium (Avogaro & de Kreutzenberg, 2005) and is associated with obesity (Yan et al., 1997). Wassman and colleagues (2004) reported that endothelial dysfunction could be induced by IL-6 through the upregulation

of an angiotensin II receptor that may contribute to the oxidative stress caused by obesity. Also, IL-6 from abdominal fat deposits has been observed to stimulate increases in CRP production in the liver and elevate plasma CRP concentrations (Niskanen et al., 2004). It has been postulated that abdominal obesity could help explain the inflammatory reaction observed in an obese population (Niskanen et al., 2004), since a significant relationship has been found between plasma CRP concentrations and BMI, total body fat mass and waist measurements (Pasceri, Willerson & Yeh, 2000). When endothelial cells are exposed to CRP, NO production is reduced due to the decreased eNOS mRNA stability and downregulation of eNOS gene expression (Ikeda, Takahashi & Shimada, 2003). In summary, obesity has various, interrelated inflammatory effects on endothelial function and NO production.

Hypercholesterolemia

Cholesterol is a well-established risk factor for coronary artery disease development (Grover-Paez & Zavalza-Gomez, 2009). Elevated blood cholesterol levels, i.e. hypercholesterolemia (Williams, Anderson & Rawson, 2013), have been correlated to abnormal endothelial function in humans (Chowienczyk et al., 1992; Creager et al., 1990; Drexler & Zeiher, 1991; Sorensen et al., 1994). Of interest, hypercholesterolemic individuals have been reported to display vasoconstriction with an intracoronary infusion of acetylecholine, which is contrary to the vasodilatory response observed in individuals with an intact endothelium (Zeiher et al., 1991). However, total serum cholesterol levels and endothelial function may not be significantly correlated (Landmesser, Hornig &

Drexler, 2000). Instead, high-density lipoprotein (HDL)-cholesterol may be an important measure in determining endothelial function in hypercholesterolemia patients, specifically for the presumed protective function of HDL-cholesterol (Tall, 1990). Moreover, the low-density lipoprotein value has less of a determining factor in the degree of endothelial dysfunction compared to the LDL/HDL ratio, indicating a possible protective effect of HDL on endothelial function (Drexler et al., 1992).

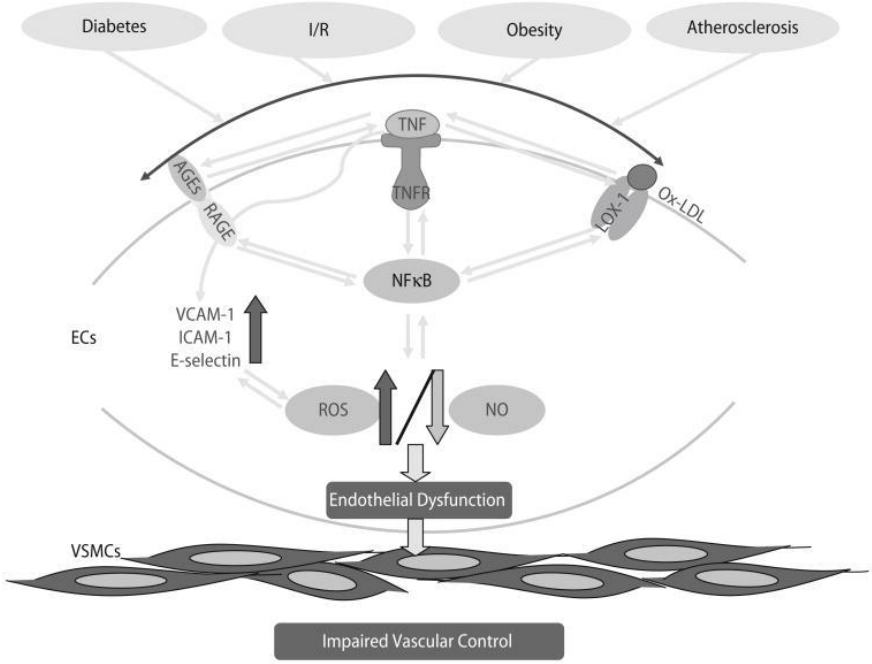
The specific mechanism by which impairment of endothelium-dependent vasodilation occurs in hypercholesterolemia individuals is mainly due to a decrease in NO bioavailability (Landmesser, Hornig & Drexler, 2000). Hypercholesterolemia has been proposed to correlate to excessive oxidative stress, specifically endothelial superoxide anion generation. The three major sources of increased superoxide anion generation in hypercholesterolemia are: the vascular NADPH oxidase, the xanthine oxidase, and the "uncoupled" NO synthase (Landmesser, Hornig & Drexler, 2000). In hypercholesterolemic rabbits, an increased vascular NADPH oxidase activity has been reported (Warnholtz et al., 1999) as well as increased circulating plasma xanthine oxidase contributing to vascular dysfunction (White et al., 1996). Also, an *in vitro* study revealed LDL could cause an uncoupling of eNOS that then generates superoxide anions (Pritchard et al., 1995). Excessive superoxide generation could not only effect NO production, but also could increase LDL oxidation and further promote endothelial cell damage through peroxynitrite and hydroxyl radical generation (O'Brien et al., 1997). Elevated plasma lipid peroxidation markers have been closely related to plasma cholesterol concentrations in hypercholesterolemic patients (Reilly et al., 1998), with

oxidized LDL level, specifically, being inversely related to endothelium-dependent vasodilation (Heitzer et al., 1996).

Research in animal models has supported the importance of antioxidants to maintain endothelium-dependent vasodilation. The administration of superoxide dismutase in hypercholesterolemic rabbits has been demonstrated to restore endothelium-dependent vasodilation (Mugge et al., 1991). Also, dietary antioxidants have been shown to preserve vessel relaxation (Keaney et al., 1993). In humans, improved endothelium-dependent vasodilation has been observed in hypercholesterolemic patients following administration of alpha-tocopherol (vitamin E) (Neunteufl et al., 1998) and ascorbic acid (vitamin C) (Ting et al., 1997). Of interest, the treatment of endothelin receptor antagonists has been reported to improve coronary endothelium-dependent vasodilation in hypercholesterolemic pigs (Best et al., 1999). Finally, the lowering cholesterol levels in patients with coronary artery disease can decrease rates of myocardial infarction and improve endothelial function (Grover Paez & Zalvalza-Gomez, 2009).

In summary, the presence of cardiovascular risk factors leads to endothelial cell disturbance, which can cause vascular remodeling and endothelial dysfunction. At the center of endothelial dysfunction is oxidative stress. Increased levels of reactive oxygen species, brought about by any number of risk factors, could cause a cascade effect that seems to focus on the interaction with NFκB. Increased activation of NFκB could decrease eNOS transcription and NO production while increasing TNFα and oxidation of LDLs. Figure 2 illustrates this interaction.

Figure 2. Indicators of Endothelial Dysfunction in Cardiovascular Diseases



Adapted from "Role of inflammatory cytokines in endothelial dysfunction" (Zhang, 2008)

Endothelial Assessment Techniques

Endothelial dysfunction undoubtedly contributes to the progression of atherosclerosis (Verma & Anderson, 2002). About 27 million individuals in North America and Europe are diagnosed with peripheral artery disease (PAD), a major manifestation of atherosclerosis (Belch et al., 2003). Therefore, the ability to accurately assess endothelial function can provide valuable clinical information and possible insight into the effectiveness of a variety of therapeutic interventions. Various techniques have been developed to evaluate endothelial activation, dysfunction, and damage (Brevetti, Schiano & Chiariello, 2008). Ultrasound assessment of FMD is the most common

technique to measure endothelial function (Corretti et al., 2002; Harris et al., 2010; Thijssen et al., 2011). Recently, L-FMC (Gori et al., 2008) and H-FMC have been proposed to increase the accuracy of endothelial function assessment.

FMD

In 1992, Celermajer et al. (1992) developed an approach to assess peripheral conduit artery diameter changes following distal limb ischemia. This technique was based on the relation between increases in blood flow, wall shear stress, endothelial NO synthases expression and NO bioactivity found in animal models (Berdeaux et al., 1994, Furchgott & Zawadzki, 1980; Tuttle et al., 2001). In humans, increases in flow-associated shear brought about by differing periods of arterial occlusion produced vasodilation in large peripheral arteries (Anderson & Mark, 1989; Sinoway et al., 1989). Subsequent studies confirmed that the FMD technique was NO dependent (Joannides et al., 1995; Lieberman et al., 1996; Mullen et al., 2001), although a majority of these studies were performed on the radial vasculature. Currently, the brachial artery is the most common site for FMD assessment (Corretti et al., 2002; Harris et al., 2010; Thijssen et al., 2011).

The FMD technique has become the most utilized and widely accepted form of non-invasive endothelium-mediated vascular reactivity assessment (Flammer et al., 2012), with over 3500 publications reporting FMD as a study outcome (Atkinson & Batterham, 2015). Utilizing an ultrasound probe, FMD assessment consists of the analysis of captured images of the brachial artery following 5-minutes of blood flow occlusion at the forearm with a sphygmomanometer cuff (Corretti et al., 2002; Harris et

al., 2011). It is important to identify clear vascular boundaries, specifically the intima to intima (Harris et al., 2010), in the longitudinal plane of the vessel (Corretti et al., 2002; Thijssen et al. 2011). Following image capturing, edge-detection and wall-tracking software is recommended for image analysis to maintain validity and reproducibility compared to a manual assessment (Thijssen et al., 2011).

Blood flow and velocity measurements are also collected during an FMD assessment via Doppler and can be used to calculate shear stress (Harris et al., 2010). It is recommended that a 60° angle between the Doppler beam and the vessel orientation is maintained along with optimal B-mode imaging to ensure measureable Doppler shifts (Thijssen et al. 2011). An increase in shear stress via reactive hyperemia has been thought to initiate eNOS production of NO, thereby causing brachial artery vasodilation (Corson et al., 1996; Pyke & Tschakovsky, 2005). Elevated levels of laminar shear stress have been observed to induce endothelial atheroprotective gene expression (Topper et al., 1996).

Historically, the important measure of reactive hyperemia has been peak FMD, which is reported as the largest artery diameter following occlusion compared to a baseline brachial artery diameter (Celermajer, et al., 1992; Corretti et al., 2002). In the original study (Celermajer et al., 1992), baseline diameter was defined as a pre-occlusion artery diameter, which has been the most frequently reported baseline in FMD literature (Lind, 2007; Pohl et al., 1986; Pyke, Hartnett & Tschakovsky, 2008; Sonka, Liang & Lauer, 2002; Thijssen et al., 2011). However, recent studies have investigated a baseline diameter during cuff inflation (Dawson et al., 2008; Magen et al., 2005; Malik,

Kondragunta & Kullo, 2008; Mullen et al., 2001; Neunteufl et al., 2000), claiming that restoration of an occlusion-induced change in vessel diameter is an important part of the FMD response itself. Although, the typical age-related reduction in FMD was not found when an occlusion baseline diameter was used (Preik et al., 2000), so a pre-occlusion brachial artery baseline diameter is traditionally recommended (Corretti et al., 2002; Thijssen et al., 2011). In the first studies using FMD, peak diameter was determined from a single frame at 60 seconds post occlusion (Celermajer et al., 1994; Celermajer et al., 1992). However, recent papers have questioned the validity of this time point for detection of a true peak diameter (Black et al., 2008; Thijssen et al., 2008). A 25-40% underestimation of true maximal FMD can result from a predetermined time point calculation (Black et al., 2008; Thijssen et al., 2008). Thus, a continuous measurement of arterial diameter response during FMD testing has been suggested (Thijssen et al., 2011) due to the differences in time to peak diameter between (Black et al., 2008; Liuni et al., 2010; Padilla et al., 2009) and within (Irace et al., 2008) groups to avoid type II statistical errors (Liuni et al., 2010; Thijssen et al., 2011).

Results from brachial artery FMD have been shown to correlate with coronary artery endothelial function and have been deemed appropriate to be used as a surrogate measure (Takase et al., 1998). In general, there is a positive correlation between endothelial health and flow-mediated vasodilation. Impaired FMD response of the brachial artery has been considered a quantitative representation of endothelial dysfunction and may be a predictor of cardiovascular events (Deanfield, Halcox & Rabelink, 2007; Yeboah, et al., 2009). Generally, FMD has been safely applied to large

groups of people across a wide age range (Celermajer et al., 1992; Leeson et al., 1997) and has also been repeated over time to measure longitudinal changes in vascular function (Quinton, Cook & Peek, 2007).

L-FMC

While FMD examines the ability of an artery to dilate in a hyperemic state, L-FMC investigates the ability of the vasculature to constrict when blood flow is attenuated. Levenson, Simon & Pithois-Merli (1987) first described an *in vivo* brachial artery vasoconstriction response to acute reduction of blood flow via a supra-systolic wrist cuff. Later, forearm cuff occlusion for 10 minutes was found to induce a significant vasoconstriction of the brachial artery due to "circulatory arrest" or "low-flow" (Anderson & Mark, 1989; Humphreys et al., 2014). Recently, a proposed method of evaluating the acute response to blood flow occlusion in the radial artery was presented by Gori and colleagues (2008). Similar to FMD methodologies, L-FMC assessment utilized ultrasound technology to image the radial artery diameter after 5 minutes of a distal, supra-systolic cuff occlusion at the wrist. However, L-FMC has also been investigated at the brachial artery during an FMD procedure (Spiro et al., 2011; Stadler, Ibrahim & Lees, 1998; Thijssen et al., 2008). The calculation of L-FMC is similar to peak FMD calculation – as a relative change in diameter in relation to a baseline diameter (Gori et al., 2008). It was postulated that a reduced diameter during conditions of low-flow could be principally linked to basal tone (Gori et al., 2008; Gori, Parker & Munzel, 2011). Also, L-FMC may complement FMD by allowing for the description of basal and

stimulated vascular function (Gori, Parker & Munzel, 2011).

The mechanisms involved in L-FMC have been thought to include increased vasoconstriction and/or decreased vasodilation factors released from the endothelium. *In vivo* evidence has been provided for L-FMC to be partly mediated though the endothelium (Dawson et al., 2012; Humphreys et al., 2014). Unlike FMD, which is an NO-mediated response, L-FMC was not altered by infusion of NO-blocker NG-monomethly-L-arginine but was affected by inhibition of EDHF and prostaglandins (Gori et al., 2008). An abolished L-FMC response of the radial artery was found with the use of an ET-1 blockade, supporting the notion of an L-FMC produced via increased vasoconstrictor stimulation (Spieker, Luscher & Noll, 2003). However, changes in plasma ET-1 levels were not found to have an effect on L-FMC at the brachial artery (Spiro et al., 2011). Thus, the understanding of the mechanisms involved in L-FMC remains unclear.

L-FMC can be measured concurrently with FMD at the brachial artery, which might provide additional and relevant information for interpretation of FMD (Gori, Parker & Munzel, 2011). There is observed heterogeneity in the response to low blood flow at the brachial artery, as healthy subjects have shown increased (Thijssen et al., 2008), decreased (Spiro et al., 2011) or no diameter changes (Stadler, Ibrahim & Lees, 1998). Weissgerber et al. (2010) examined L-FMC at the brachial and radial artery of pregnant and non-pregnant women and confirmed the presence of L-FMC at the radial artery, but no such response at the brachial artery. The heterogeneity of artery responses to low-flow has some implications for L-FMC use as a surrogate for the coronary

arteries. However, those with CVD risk factors have displayed both diminished (Harrison et al., 2011) and improved (Stadler, Ibrahim & Lees, 1998) L-FMC responses at the brachial artery. Interestingly, the use of a composite end point (FMD + L-FMC) at the radial artery has been shown to improve detection of endothelial dysfunction in already diagnosed patients (Gori et al., 2010). This limited and conflicting evidence requires further research to understand the underlying stimuli of L-FMC and quantify the heterogeneity between arteries so correct interpretation of L-FMC can be performed.

H-FMC

During an FMD procedure, an increase in shear stress via reactive hyperemia is experienced following 5-minutes of blood flow occlusion (Corretti et al., 2002; Harris et al., 2010; Thijssen et al., 2011). Immediately following occlusion cuff release, some arteries have displayed a constriction response to increased blood flow (Dobrosielski et al., 2006; Jiang et al., 2011), termed high-flow-mediated constriction (H-FMC). This opposing vasoconstrictor response conceals a shear stress-induced NO-mediated dilation of the artery (Dobrosielski et al., 2006; Jiang et al., 2011). A concealed or blunted dilatory response to shear stress could be interpreted as a marker of endothelial dysfunction. It is postulated that the observed vasoconstriction could result from an increase in ET-1 release and/or decrease in transmural pressure (Dobrosielski et al., 2006; Jiang et al., 2011; Kuchan & Frangos, 1993). A structural difference, i.e. degree of arterial stiffness, has also been postulated (Dobrosielski et al., 2006). Currently, limited research has investigated this constriction phenomenon following blood flow occlusion at

the brachial (Dobrosielski et al., 2006) and radial artery (Jiang et al., 2011).

Endothelial Assessment Analysis

Allometric Scaling

Since the technique of brachial artery FMD was first introduced (Celermajer et al., 1992), most of the 3500 publications have reported a conventional representation of FMD – as %Δ and/or mmΔ (Atkinson & Batterham, 2015; DeVan et al., 2013). However, several scaling and normalization adjustments have been suggested to improve sensitivity, accuracy and significance of FMD data (DeVan et al., 2013). Since FMD% is calculated as the difference between peak diameter and baseline diameter divided by baseline diameter and multiplied by 100, it is considered a ratio of peak diameter and baseline diameter (Atkinson et al., 2013). A ratio-approach to size-scaling a biological change is argued to not be appropriate (Atkinson & Batterham, 2013; Atkinson & Batterham, 2015; Packard & Boardman, 1999). Allometric scaling of vessel baseline diameter has been recently suggested to improve specificity of FMD for endothelial function and negate a ratio-approach of FMD reporting (Atkinson & Batterham, 2013; Atkinson et al., 2013).

Allometric scaling of baseline vessel diameter consists of logarithmically transforming the baseline diameter and peak diameter values, and deriving the change in diameter on the logged scale (Atkinson & Batterham, 2013). Then, the slope of the regression between the logarithmically transformed baseline diameter and peak diameter is calculated. If the slope is less than 1, peak diameter does not increase as a constant of

baseline diameter and inferences about differences in endothelial function based on FMD% might be unreliable (Atkinson & Batterham, 2013). If this is the case, diameter changes on the logged scale are entered into an ANCOVA model, with change in diameter as the outcome and a log-transformed baseline diameter as a covariate (Atkinson & Batterham, 2013; Atkinson et al., 2013). Covariate-adjusted means for diameter change are then back-transformed to provide a baseline-adjusted ratio of peak diameter and baseline diameter. An adjusted percentage change, or "corrected FMD%" could then be reported by taking the baseline-adjusted changes in diameter and subtracting a value of 1 and then multiplying by 100 (Atkinson & Batterham, 2013; Atkinson et al., 2013). This new approach to size-scaling the FMD response has been found to alter study inferences (Atkinson & Batterham, 2013; Atkinson & Batterham, 2013).

There has been substantial debate over allometric scaling of FMD measurements. While some agree with the premise of the argument of Atkinson and Batterham (Stoner, Faulkner & Sabatier, 2013; Woodman & Mangoni, 2013), there are several factors to consider. First, the criteria for allometric scaling are not consistently met (DeVan et al., 2013). For intervention and repeated measures studies, FMD is measured in the same way and adjusting for baseline will matter little, since any incurred bias should be balanced out within each individual across the study (Woodman & Mangoni, 2013). Since FMD research does not consistently meet the criteria for allometric scaling, it could be argued that allometric scaling provides little additional value. Second, allometric scaling of baseline diameter has been discovered to weaken the relationship between

FMD response and CVD large study populations, specifically in the Multi-Ethnic Study of Atherosclerosis (Atkinson & Batterham, 2013). This lack of relationship between FMD response and CVD is argued to miss a possible physiological link between larger vessels, low FMD response, and CVD (Mheid & Quyyumi, 2013). Third, there is no evidence that scaling the FMD values is predictive of incident events or diseases, but conventional brachial artery FMD values does have empirical evidence (DeVan et al., 2013; Seals, Jablonski & Donato, 2011; Yeboah et al., 2009).

Finally, Atkinson and Batterham (2013) present additional "corrected FMD" results to Celermajer and colleagues (1992) original research from differing vascular beds and reported equivalent FMD% across vascular beds. However, the assumption that differing vascular beds should provide equivalent FMD% (Atkinson & Batterham, 2013) may be misleading if there are vascular differences in structural composition, vasomotor tone, shear stress and endothelial function (Stoner, Faulkner & Sabatier, 2013). Interestingly, arterial wall structural differences are present in varying parts of the vasculature (Caro, 2012). Vasomotor tone is affected by transmural pressure and shear stress (Kelm & Schrader, 1990) and upper and lower limbs experience differing degrees of hydrostatic pressure and shear stress at rest (Newcomer et al., 2005). Also, endothelial function has been reported to be unequal between vascular beds (Newcomer et al., 2005; Thijssen et al., 2011; Stoner et al., 2006).

Allometric scaling may prove to be an important addition to the traditional approach of FMD reporting. Currently, adopting an allometrically scaled FMD in conjunction with traditional FMD measures for cross-sectional studies could be

considered. The potential for enhanced sensitivity and validity of FMD with allometric scaling requires continuing research.

Summary

The endothelium produces essential factors involved in vasomotor control. The disruption and disturbance of the endothelium and endothelial-derived factors can cause vascular remodeling and endothelial dysfunction via oxidative stress. Cardiovascular risk factors, such as obesity and hypercholesterolemia, can exacerbate the development of endothelial dysfunction and atherosclerosis. To detect and quantify endothelial function, FMD and L-FMC techniques have been proposed and developed. FMD via reactive hyperemia has been deemed the most accepted and utilized non-invasive technique for assessing endothelial function in healthy and at-risk populations. Historically, FMD has evaluated the peak dilatory response of an artery compared to a baseline diameter measure, while recent studies have quantified L-FMC and proposed new ways to scale the FMD response. However, studies investigating the H-FMC response to reactive hyperemia are lacking. The following dissertation evaluates the H-FMC response to reactive hyperemia in children, adolescents and adults and examines the reproducibility of the H-FMC response in young adults.

CHAPTER 3. INTRA- AND INTER-DAY REPRODUCIBILITY OF HIGH-FLOW-MEDIATED CONSTRICTION RESPONSE IN YOUNG ADULTS

Intra- and Inter-day Reproducibility of High-Flow Mediated Constriction Response in Young Adults

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Summary

Purpose: Previously, we have demonstrated that high-flow-mediated constriction (H-FMC) of the brachial artery has been shown to negatively affect flow-mediated dilation (FMD). However, the reproducibility of an H-FMC response is unknown. The aim of this study was to determine the intra- and inter-day reproducibility of H-FMC in young adults.

Methods: Thirty young adults (15 male, 15 female; 24±3 years) were assessed for H-FMC reproducibility via high-resolution ultrasound imaging of the brachial artery during and after forearm occlusion of a normal FMD procedure. Two vascular assessments were conducted for all participants during two separate visits with a minimum of 7-days in between. H-FMC was characterized as the greatest 10-second average constriction occurring after three seconds post occlusion compared to baseline brachial artery diameter and considered present if the percent change was less than -0.1%.

Results: Of the 120 total vascular assessments performed in this study, 98 of the assessments (80.3%) displayed an H-FMC. H-FMC diameter was not statistically different for intra-day comparisons for visit 1 (P=0.39) or 2 (P=0.55) or inter-day comparisons between the first (P=0.61) or second (P=0.10) assessments. H-FMC percentage was also not statistically different for intra-day comparison for visit 1 (P=0.94) or 2 (P=0.15) or inter-day comparisons between the first (P=0.63) or second (P=0.16) assessments.

Conclusion: These data are supportive of H-FMC being reproducible in young adults and included in future FMD studies. The impact of H-FMC on future CVD risk and development warrants evaluation.

Introduction

Endothelial dysfunction is a precursor in the development of atherosclerosis and cardiovascular disease (CVD) risk (Ross, 1999; Vita & Keaney Jr, 2002; Bonetti, Lerman & Lerman, 2003; Ras et al., 2013). A widely used method of assessing endothelial function is flow-mediated dilation (FMD) (Celermajer et al., 1992; Corretti et al., 2002; Ras et al., 2013), which utilizes ultrasound imaging of the brachial artery following acute reactive hyperemia. FMD has been inversely associated with future CVD events (Brevetti et al., 2003; Katz et al., 2005; Muiesan et al., 2008; Ras et al., 2013). Recently, during FMD measurement in the brachial artery, others and we have reported a biphasic response to reactive hyperemia, in which a vessel constriction phase occurs prior to a dilatory phase (Dobrosielski et al., 2006; Ostrem et al., 2015, Ostrem et al., 2016). This constriction phase during reactive hyperemia has been termed high-flow-mediated constriction (H-FMC).

Presently, we have reported approximately 67% of children and adolescents and 69% of adults display an H-FMC during FMD assessment prior to a shear stress-mediated dilation of the brachial artery (Ostrem et al., 2015; Ostrem et al., 2016). The presence of an H-FMC significantly reduced FMD response of the brachial artery to hyperemia compared to individuals without an H-FMC response when using a baseline diameter calculated during blood flow occlusion (Ostrem et al., 2015; Ostrem et al., 2016). However, the reproducibility of brachial artery H-FMC has not been described. For H-FMC to be of value in assessing vascular function and as a potential biomarker of CVD it must be shown to be reproducible. Therefore, the aim of this study was to

determine the intra- and inter-day reproducibility of H-FMC in healthy young adults (aged 18-30). We hypothesized that H-FMC in young adults will have an intra- and inter-day reproducibility similar to the reported reproducibility of FMD in the brachial artery (Sorensen et al., 1995).

Materials and Methods

Study Population

Thirty adults (15 males, 15 females) were assessed for the reproducibility of an H-FMC. Subjects were recruited from the University of Minnesota and surrounding community and all subjects were free of known disease. The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board and all participants gave written informed consent. The procedures followed were in accordance with the institutional review board and HIPAA guidelines. A total of four vascular assessments were performed per participant – two vascular assessments conducted during each of two separate visits (30 minutes: time between assessments each day), which were separated by a minimum of 7-days. Vascular assessments for each of the two visits were conducted during the same time of day, to avoid diurnal variation (Jarvisalo et al., 2006). Participants were instructed to fast for a minimum of 6 hours prior to each visit. It was recommended to ingest a similar meal prior to fasting for each of the two visits. Subjects were required to refrain from alcohol or caffeine ingestion within 6 hours prior to testing as well as avoid strenuous exercise or physical activity 24 hours prior to the study visit.

Physical Assessments

Measurements for height and weight were obtained with a standard stadiometer and electronic scale (Cardinal Detecto, Model 758C, Webb City, MO, USA). Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters-squared (m^2).

Vascular Assessments

Vascular testing was performed in the Vascular Laboratory in the Human Performance and Teaching Laboratory (HPTL) at the University of Minnesota. Subjects were tested in a quiet, climate-controlled room (22-23°C) by a single sonographer. Resting blood pressure was recorded using an automated sphygmomanometer (Colin BP-8800, Colin Medical Instruments Corp., San Antonio, TX, USA) on the right arm prior to vascular assessment. A rapid-inflating occlusion cuff (D.E. Hokanson, Inc., Bellevue, WA) was also placed approximately five centimeters (cm) distal to the antecubital space on the left forearm. Following 10 minutes of quiet rest in the supine position, vascular images of the left brachial artery were obtained proximal to the antecubital fossa in the longitudinal plane using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with a 8-15 MHz linear array probe held at a constant pressure on the skin and at a fixed point over the imaged artery by a stereotactic arm.

Following resting vascular imaging, the Hokanson occlusion cuff on the left forearm was subsequently inflated to a suprasystolic pressure level of 200 mmHg and

maintained for 5 minutes. Vascular images were captured 20-seconds prior to cuff release until 3 minutes post-cuff release and were digitized and stored on a personal computer for later off-line analysis using an electronic wall-tracking software program (Vascular Research Tools 6, Medical Imaging Application, LLC, Iowa City, IA, USA). All subjects remained supine throughout the 30-minute interim between the first and second vascular assessment. The second vascular assessment was initiated with inflation of the left forearm cuff and conducted exactly as the first. Vascular images were analyzed for brachial artery baseline diameter, peak shear, maximal blood flow and brachial artery constriction. The baseline brachial artery measurement was defined as a 10-second average just prior to release of the blood pressure cuff, i.e., occlusion baseline, to negate any low-flow mediated constriction that may occur during occlusion (Gori et al., 2008). Peak shear was used to estimate shear stress as a 10-second average during post occlusive reactive hyperemia, which was calculated as blood flow velocity (*q)* divided by arterial diameter (D). Maximal flow (m/s) was defined as the largest 10-second average rate of blood flow following cuff release. H-FMC was observed and characterized using a 10-second average of the lowest point, (i.e. H-FMC diameter), from baseline brachial artery diameter following 3-seconds post cuff release and considered present if the percent change was less than -0.1% (H-FMC percentage) (Figure 1). All vascular analysis and interpretation was performed by a trained investigator who was blinded to study subjects and conditions. Image analysis and calculation of H-FMC was conducted for each subject once the final visit was completed to prevent biased interpretation.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Demographic characteristics for the population and vascular measurements are expressed as mean ± standard deviation (SD). Independent t-tests were conducted on gender differences for physical characteristics and blood pressure and heart rate measures. Paired t-tests were used to compare brachial artery baseline diameter, H-FMC measures, peak shear and maximum flow within each visit and between visit 1 and 2. A repeated measures ANOVA was conducted among all four vascular assessments for baseline diameter and H-FMC measurements. Greenhouse-Geisser F-statistic and significance were reported for within-subjects effects when statistical significance of assumed sphericity was present. An alpha value of 0.05 was denoted as statistically significant.

Reproducibility of H-FMC was also assessed using a two-way agreement intraclass correlation coefficient (ICC) and coefficient of variation (CV) along with mean variation. Intraclass correlation coefficient comparisons were conducted between each of the two visits and within each visit for H-FMC diameter. Bland-Altman analyses were also performed to display the agreement between and within day for H-FMC diameter in our study population.

Results

Mean demographic characteristics of young adults are presented in Table 1. The cohort ranged in age from 19 to 29 years old. As expected males had significantly greater

height, weight, BMI, systolic blood pressure (SBP), and heart rate compared to females. Of the 120 total FMD assessments performed in this study, 97 of the assessments (80.8%) displayed an H-FMC. Twelve subjects displayed an H-FMC during every FMD assessment and 15 subjects had an H-FMC in three of the four assessments.

Intra- and inter-day comparisons for variables of interest are included in Table 2. No statistically significant difference was found for any variable when intra- and inter-day analysis was performed. Brachial artery baseline diameter was not different for intra-day measures for visit 1 (P=0.36) and 2 (P=0.26). Inter-day measures of baseline diameter between the first (P=0.72) or second (P=0.06) procedures were also not significantly different. A repeated measures ANOVA produced no statistically significant variation for baseline diameter (P=0.24).

Intra-day comparisons for peak shear were not significantly changed for visit 1 (P=0.33) and 2 (P=0.47) or inter-day between the first (P=0.96) or second (P=0.87) assessments. Likewise, maximal flow was not different for intra-day measures within visit 1 (P=0.24) and 2 (P=0.36) or inter-day comparison between the first (P=0.89) or second (P=0.94) procedures.

H-FMC diameter was not changed for intra-day measurements within visit 1 (P=0.39) and 2 (P=0.55). Inter-day measures of H-FMC diameter between the first (P=0.61) or second (P=0.10) procedures were also not significantly different. No statistically significant variation for H-FMC diameter was found when a repeated measures ANOVA was performed (P=0.334). H-FMC percentage was not significantly changed during intra-day comparison for visit 1 (P=0.94) or 2 (P=0.15) or inter-day

comparisons between the first (P=0.63) or second (P=0.16) assessments. No significant variation for H-FMC percentage was found when a repeated measures ANOVA was performed (P=0.511).

The mean intra-day variation of H-FMC was -0.24±1.82%. The mean inter-day variation of H-FMC was -0.08±1.63%. The overall reproducibility of intra- and inter-day H-FMC diameter were ICC=0.98, CV=2.77% and ICC=0.96, CV=3.63%, respectively. These results as well as visual representation of the study protocol format are displayed in Figure 2. Bland-Altman plots (Figure 3) display acceptable agreement for intra- and inter-day H-FMC diameter assessment in our study population.

Discussion

The aim of the present study was to determine the intra- and inter-day reproducibility of an H-FMC during vascular assessment in healthy, young adults. This study demonstrated that H-FMC is highly reproducible both intra- and inter-day. Also, peak shear and maximal flow, the primary stimuli for vasodilation, (Busse & Fleming, 1998; Davies, 1995; Dimmeler et al., 1999; Kuchan & Frangos, 1994; Rubanyi, Romero & Vanhoutte, 1986) are consistent throughout intra- and inter-day vascular assessments.

H-FMC diameter had greater variation (overall CV = 3.2%) compared to the reported reproducibility of FMD by Sorensen and colleagues (1995) (overall CV = 1.8%). However, the reported CV for H-FMC diameter in this study is still very good when compared to other FMD reproducibility studies (overall CV = 12.2%) (Ghiadoni et al., 2012; Hijmering et al., 2001). Also, H-FMC had very good reproducibility when

including an ICC (overall ICC = 0.97), which was not reported in the FMD reproducibility study by Sorensen and colleagues (1995) but was similar to other reported correlation coefficients of FMD (ICC = 0.914) (Ghiadoni et al., 2012). Considering the intra- and inter-day ICC and CV of H-FMC diameter, an argument could be made to include the absolute vessel measure to quantify vascular change longitudinally and assess cross-sectional vascular health. H-FMC diameter may provide significant value in vascular health assessment similar to brachial artery baseline diameter or FMD, which has been inversely associated with CVD status (Yeboah et al., 2009).

Since vessel constriction and dilation percentage is contingent on the vessel baseline diameter (Corretti et al., 2002; Harris et al., 2010), it would be beneficial to establish a universal timeframe for brachial artery baseline measurement during FMD assessment. Still, there is conflicting evidence concerning the effect of baseline selection on reported FMD (Thijssen et al., 2008; Ostrem et al., 2015; Ostrem et al., 2016). Historically, a pre-occlusion baseline has been utilized to calculate vessel response to reactive hyperemia (Corretti et al., 2002; Thijssen et al., 2011). However, the current study has demonstrated a high reproducibility of the post-occlusion vessel diameter. Theoretically, consideration of a post-occlusion diameter when determining peak FMD would be logical if the goal is to assess the total shear stress-mediated vessel change, since the post occlusion baseline may be the smallest brachial artery diameter in a majority of individuals. Therefore, the use of a post-occlusion baseline may give a better indication of true dilation capacity. However, the absolute diameter change from

occlusion baseline, or pre-occlusion baseline, to H-FMC could also be an ancillary measure of endothelial health.

Currently, it is unknown if, and to what degree, H-FMC is correlated with the development of atherosclerosis and cardiovascular disease. Previously, individuals displaying an H-FMC have significantly lower FMD measures compared to Non-H-FMC individuals (Ostrem et al., 2015; Ostrem et al., 2016). Historically, a reduced FMD response is considered a sign of endothelial dysfunction, as seen in individuals with greater severity of coronary artery stenosis (Kaku et al., 1998). However, Dobrosielski and colleagues (2006) reported that older males had less post-occlusion brachial artery constriction compared to young males. Therefore, H-FMC may be an indicator of vessel distensibility, reflecting mainly the elasticity of the vessel (Hoeks et al., 1990). Considering such a large number of children, adolescents and adults have displayed an H-FMC in previous studies (Dobrosielski et al., 2006; Ostrem et al., 2015; Ostrem et al., 2016) in conjunction with the current study's healthy young adult population, it could be postulated that an H-FMC may serve as an ancillary representation of healthy endothelial function and increased vessel distensibility. Thus, it may be beneficial to assess H-FMC in a longitudinal cohort to fully comprehend the correlation with atherosclerosis and cardiovascular disease.

There are some limitations to the present study. Only 10% of the study population (3 individuals) was consistently classified as Non-H-FMC individuals. This is contrary to previously reported frequencies in a cross-sectional study for an adult population (31%) (Ostrem et al., 2016). However, this discrepancy is most likely due to a disparity in

sample size between the previous and current studies. Also, menstrual cycle phase was not controlled for in women; however, this could only affect inter-day reproducibility and, theoretically, would not affect intra-day reproducibility.

In conclusion, H-FMC presence and diameter are very reproducible both intra- and inter-day in young adults. The findings of this study further support consideration of an extended time period of analysis during FMD technique, one that includes the response of the artery immediately following occlusion. Constriction diameter and H-FMC may be relevant not only in the comparison between various groups of individuals but also longitudinal studies interested in treatment or examination of CVD development.

TABLE LEGENDS

Table 1. Physical and Vascular Characteristics

Table 2. Intra- and Inter-day Comparisons for Vessel Diameter and Blood Flow

Table 1. Physical and Vascular Characteristics

	Male	Female	P-value
N	15	15	
Age, years	24±3	24±3	0.525
Height, cm	180.3±5.9	166.9±7.1	<0.001
Total Body Mass, kg	81.2±14.5	60.2±12.6	<0.001
BMI, kg/m^2	24.9±3.6	21.5±3.6	0.016
Waist Circumference, cm	81.5±8.1	69.6±10.0	0.002
SBP, mmHg	118±10	109±7	<0.001
DBP, mmHg	64±7	63±6	0.186
Heart Rate, bpm	55±8	55±9	0.908

Note: Values are means±SD;
BMI – Body Mass Index
SBP – Systolic Blood Pressure
DBP – Diastolic Blood Pressure

Table 2. Intra- and Inter-day Comparisons for Vessel Diameter and Blood Flow

	Visit 1		Visit 2	
	Test 1	Test 2	Test 1	Test 2
Baseline Brachial Artery Diameter, mm	3.70±0.67	3.73±0.70	3.68±0.65	3.64±0.66
H-FMC Diameter, mm	3.66±0.67	3.69±0.69	3.63±0.64	3.61±0.66
H-FMC, %	-1.12±1.74	-1.14±1.18	-1.29±1.31	-0.80±1.50
Peak Shear, sec^{-1}	225.2±65.7	215.5±75.6	225.9±79.7	217.5±78.4
Maximal Flow, m/s	0.80±0.19	0.76±0.18	0.79±0.22	0.76±0.21

Note: Values are means±SD; * denotes <0.05 significance

H-FMC – High-Flow-Mediated Constriction

Figure Legend

Figure 1. A theoretical representation of a Flow-Mediated Dilation (percent change in brachial artery diameter from baseline) response in High-Flow-Mediated Constriction (♦) and Non-High-Flow-Mediated Constriction (●) adults.

Figure 2. Study protocol, High-Flow-Mediated Constriction diameter reproducibility.

Figure 3. Bland-Altman plots for intra-day (A,B) and inter-day (C,D) difference in High-Flow-Mediated Constriction diameter in young adults.

Figure 1

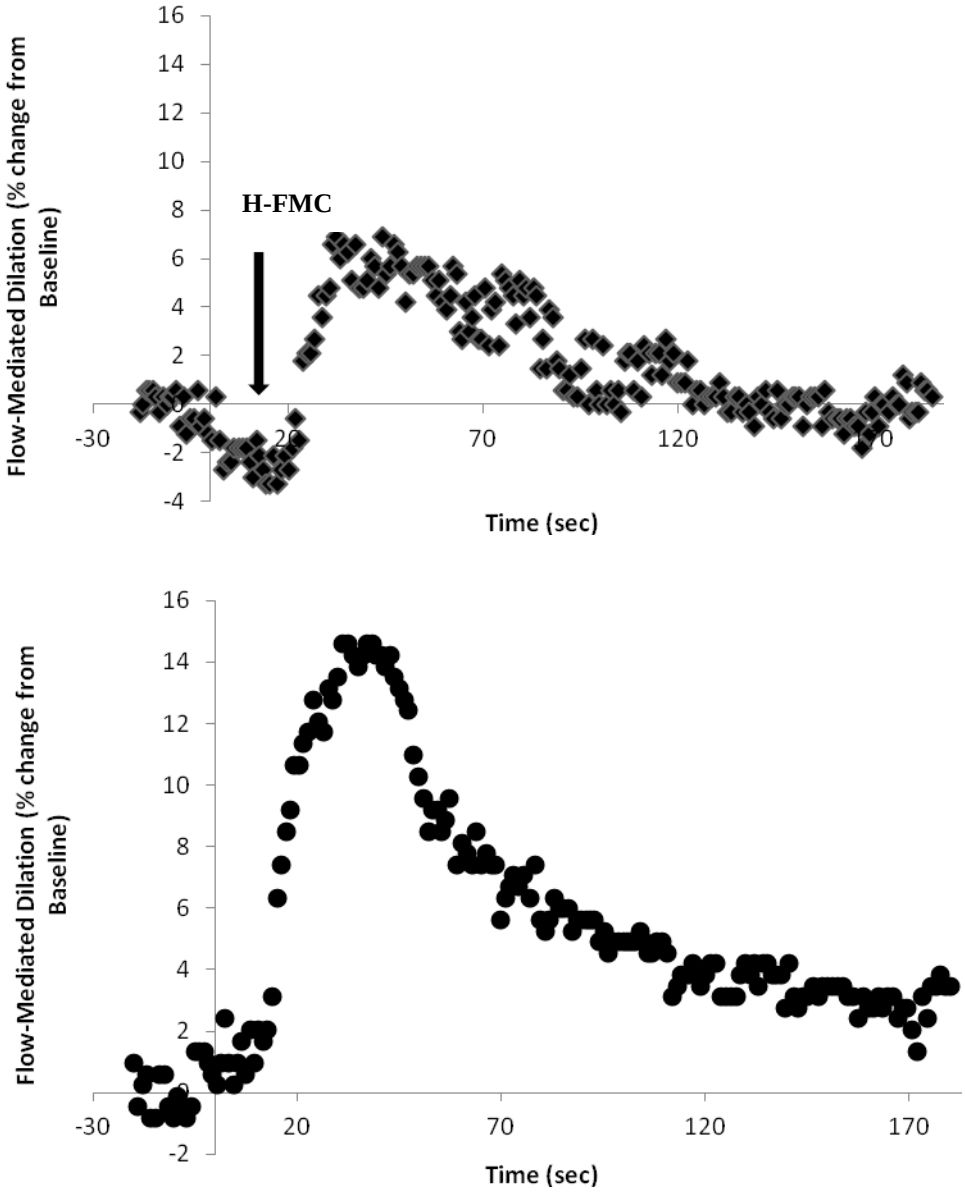

Flow-Mediated Dilation (% change from Baseline)
H-FMC
Time (sec)
Flow-Mediated Dilation (% change from Baseline)
Time (sec)

Figure 2

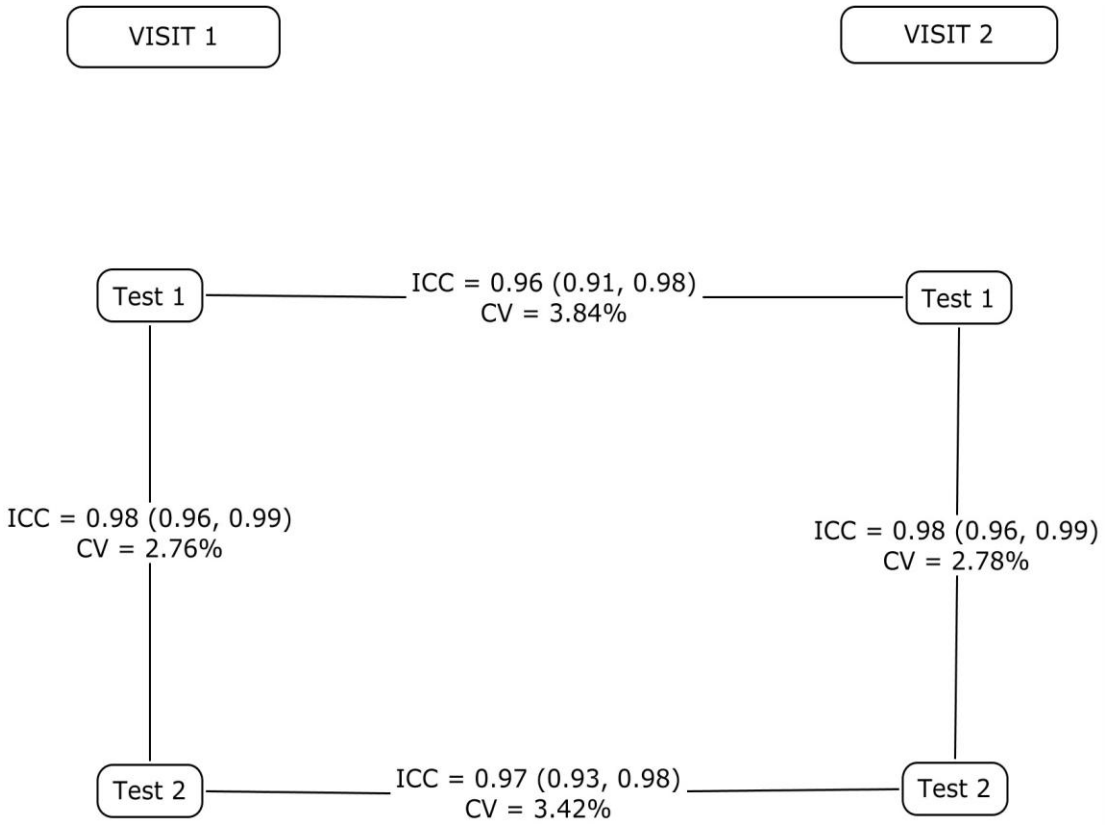
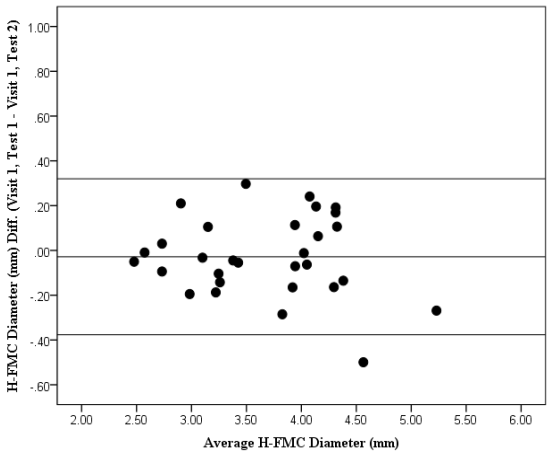
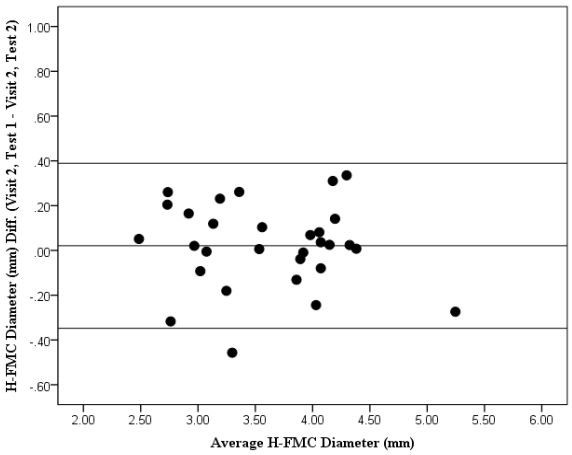

VISIT 1
VISIT 2
Test 1
ICC = 0.96 (0.91, 0.98)
CV = 3.84%
Test 1
ICC = 0.98 (0.96, 0.99)
CV = 2.76%
ICC = 0.98 (0.96, 0.99)
CV = 2.78%
Test 2
ICC = 0.97 (0.93, 0.98)
CV = 3.42%
Test 2

Figure 3

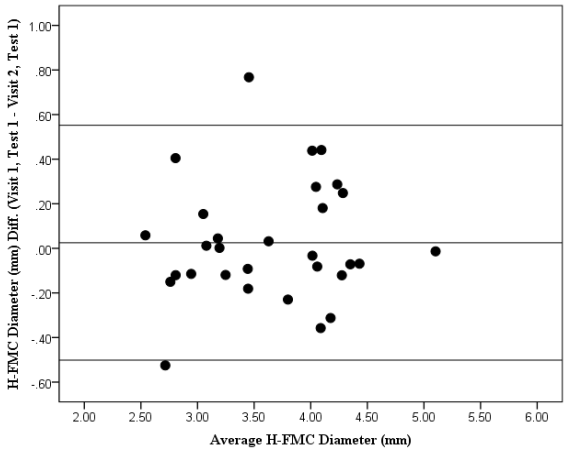
A



B



C



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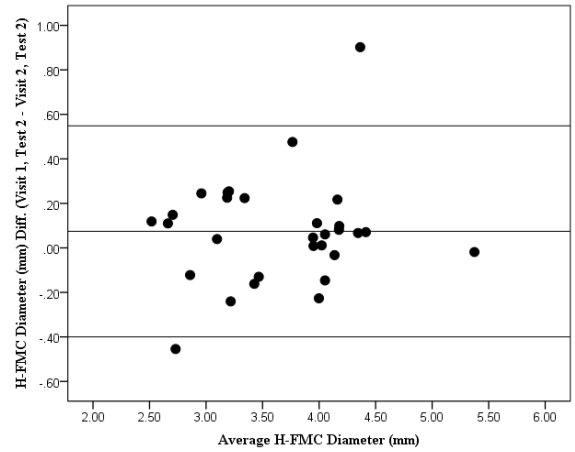

CHAPTER 4. PRESENCE OF A HIGH FLOW-MEDIATED CONSTRICTION PHENOMENON PRIOR TO FLOW-MEDIATED DILATION IN NORMAL WEIGHT, OVERWEIGHT AND OBESE CHILDREN AND ADOLESCENTS

Presence of a High Flow-Mediated Constriction Phenomenon Prior to Flow-Mediated Dilation in Normal Weight, Overweight and Obese Children and Adolescents

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Short Title: H-FMC and FMD in Children and Adolescents

Key Words: Ultrasound, Reactive Hyperemia, Flow-Mediated Dilation, Flow-Mediated Constriction, Adolescents

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SUMMARY

Purpose: When assessing vasomotor endothelial function by reactive hyperemia, the brachial artery, in some individuals, constricts immediately before beginning to dilate following cuff release. We call this response high-flow-mediated constriction (H-FMC). The aim of this study was to describe the frequency of the H-FMC during reactive hyperemia in children and adolescents throughout a range of body mass index (BMI) values, and to investigate differences in flow-mediated dilation (FMD), cardiovascular, and anthropometric measures between subjects with and without H-FMC.

Methods: FMD was assessed in 136 children and adolescents (61 male, 75 female; 13±3 years) by sonographic imaging of the brachial artery. H-FMC was characterized as the lowest point from the baseline brachial artery diameter immediately following reactive cuff release. Independent t-tests were used to compare subjects with and without H-FMC.

Results: H-FMC was observed in 91 of the 136 participants (66.9%). No significant difference was found between H-FMC and non-H-FMC subjects for age (P=0.602), gender (P=0.767), height (P=0.227) or weight (P=0.171). BMI percentile was nonsignificantly higher (91.8^{th}±14.9^{th}vs.84.6^{th}±22.8^{th} percentile, P=0.057) and FMD was significantly lower (5.43±3.41vs.8.05±3.97%, P<0.001) in H-FMC than in non-H-FMC subjects. Adding H-FMC to FMD produced no significant difference between H-FMC and non-H-FMC individuals (8.03±3.27%vs.8.05±3.97%, P=0.977).

Conclusion: Approximately 67% of participants demonstrated an H-FMC during reactive hyperemia. BMI percentile was nonsignificantly higher and FMD was significantly lower in children and adolescents who displayed this phenomenon.

INTRODUCTION

Flow-mediated dilation (FMD) is widely used as a noninvasive method for measurement of endothelial function through sonographic imaging of the brachial artery following reactive hyperemia (Corretti et al., 2002). Endothelial dysfunction has been associated with cardiovascular events (Deanfield, Halcox & Rabelink, 2007) as well as increasing age (Celermajer et al., 1994), gender (Kapuku et al., 2004; Juonala et al., 2008), cigarette smoking (Lekakis et al., 1998), and obesity (Tounian et al., 2001; Williams et al., 2005). An increase in shear stress via increased flow has been thought to initiate endothelial nitric oxide (NO) synthase production and subsequent release of NO (Corson et al., 1996; Pyke & Tschakovsky, 2005). A delayed arterial dilatory response to shear stress has been observed in radial (Jiang et al., 2011), brachial (Corretti et al., 2002) and femoral (Kooijman et al., 2008) vascular beds. In the radial artery, a relatively recent study (Jiang et al., 2011) concluded that, during high blood flow, an opposing vasoconstrictor stimulus conceals the shear stress-induced NO-mediated dilation. It was postulated that vasoconstriction could result from the decrease in transmural pressure produced by the sudden fall in downstream flow resistance and/or by a shear stress-stimulated constrictor release such as endothelin-1 (Jiang et al., 2011; Kuchan & Frangos, 1993).

A delayed dilation response is regularly observed in humans (Corretti et al., 2002; Corretti, Plotnick & Vogel, 1995; Black et al., 2008). However, immediately following the cuff release, the arteries of some individuals constrict before they begin to dilate. This physiological response has been referred to as a flow-mediated constriction (FMC)

(Figure 1) (Jiang et al., 2011; Rubanyi & Gabor, 1995; Turner, 2011; Gori et al., 2008). An alternative method for assessing the vascular function has been proposed by measuring the radial artery constriction during distal occlusion (Gori et al., 2008). This procedure has been termed low-flow-mediated constriction (L-FMC) (Gori et al., 2008). In contrast, a high-flow-mediated constriction (H-FMC) has been observed in healthy adults at the radial artery following occlusion (Jiang et al., 2011). However, we found no report in the literature regarding the occurrence of brachial artery H-FMC in children and adolescents. The aim of the present study was to assess the rate of brachial artery H-FMC during reactive hyperemia in children and adolescents throughout a large range of body mass index (BMI) values, and to investigate differences in FMD, and hemodynamic and anthropometric measures, between those subjects who experience this phenomenon and those who do not.

MATERIALS AND METHODS

Study Population

One hundred thirty-six children and adolescents (61 males, 75 females) were assessed for peak FMD. Subjects were recruited from a pediatric weight management clinic (patients and siblings) and from the community via flyers and advertisements. The study protocol was reviewed and approved by the University of Minnesota Institutional Review Board and all participants, along with parents/guardians, gave written informed assent and consent. The procedures followed in the study were in accordance with the institutional review board and HIPAA guidelines. Subjects were fasted for at least 8 hours prior to vascular assessment and were asked to abstain from caffeine for at least 4

hours on the morning of testing. Avoidance of strenuous exercise or physical activity was also required for 24 hours prior to the study visit.

Physical Assessments

Measurements for height and weight were obtained with a standard stadiometer (Avrton, Model S100, Prior Lake, MN) and electronic scale (ST Scale-Tronix, Serial No. 5022-8893, White Plains, NY), respectively. BMI was calculated as weight in kilograms divided by height in meters-squared. Tanner stage was assessed by a trained pediatrician or registered nurse. Waist and hip circumferences (in centimeters) were obtained with a Gulick measuring tape (Creative Health Products, Ann Arbor, MI).

Vascular Assessments

Vascular testing was performed in the Vascular Biology Laboratory in the Clinical and Translation Science Institute at the University of Minnesota. Subjects were tested in a quiet, climate-controlled room (22-23°C). Resting blood pressure was recorded using an automated sphygmomanometer (Colin Medical Instruments Corp., San Antonio, TX) on the right arm prior to FMD assessment. A blood pressure cuff was also placed on the left forearm. Following 15 minutes of quiet rest in the supine position, vascular images of the left brachial artery were obtained proximal to the antecubital fossa in the longitudinal plane using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA) with a 8-15 MHz linear

array probe held at a constant pressure on the skin and at a fixed point over the imaged artery by a stereotactic arm.

The blood pressure cuff on the left forearm was inflated to a suprasystolic pressure level of 200 mmHg and maintained for 5 minutes. Vascular images were captured 20 seconds prior to cuff release until 3 minutes post-cuff release and were digitized and stored on a personal computer for later off-line analysis using an electronic wall-tracking software program (Vascular Research Tools 5, Medical Imaging Application, LLC, Iowa City, IA). Vascular images were assessed and baseline brachial artery measurements were recorded as a 10-second average just prior to blood pressure cuff release (i.e. occlusion baseline), and a 10-second average immediately following blood pressure cuff release. Peak dilation during each study was defined as the 10-second average of the greatest percent change from baseline brachial artery diameter. Shear rate was used to estimate shear stress and calculated as blood flow velocity divided by arterial diameter. The maximal flow 10-second average blood flow after cuff release was reported as maximal flow. The H-FMC was characterized using a 10-second average of the lowest point from baseline brachial artery diameter following cuff release and considered present if the diameter increased by less than -0.1% or decreased (Figure 1). A trained sonographer performed all digital sonographic image analysis. When FMD was measured 7 days apart, the coefficient of variation was 11.1% in our laboratory, demonstrating good reproducibility.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 21 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0; IBM Corp., Armonk, NY). Descriptive statistics were conducted for the two groups on anthropometric and hemodynamic measurements. Independent t-tests were used to compare differences between subjects who demonstrated an H-FMC and those who did not. A correlation matrix between analyzed parameters was implemented among significant anthropometric and vascular measures. A one-way analysis of variance was conducted for the degree of H-FMC for BMI percentile**.** An α value of 0.05 was denoted as statistically significant.

RESULTS

Mean demographic characteristics of children are presented in Table 1. Of the 136 children and adolescents examined in this study, 91 (66.9%) displayed an H-FMC. Children and adolescents ranged in age from 8 to 17 years old, with a mean age of 13±3 years. When classified by BMI percentile, 70.6% of the study population was considered obese, 9.5% overweight and 19.9% were normal weight for their age and gender. When grouped by the presence or absence of H-FMC according to BMI percentile, 70.8% of obese and 76.9% of overweight children and adolescents displayed an H-FMC versus 48.1% of normal weight children and adolescents. Comparing BMI percentile within the H-FMC group and degree of H-FMC, average constriction was significantly greater in normal weight individuals than in both overweight and obese combined (-3.53±1.88% vs. -2.45±1.59%, P=0.03). No differences were observed for composition of gender, levels of systolic and diastolic blood pressure, heart rate, height, weight, BMI, Tanner stage, or hip

and waist circumference (Table 1). BMI percentile was nonsignificantly higher in H-FMC than in non-H-FMC children and adolescents (Table 1). Table 2 displays the vascular measures for the study population. Baseline brachial artery diameter, average shear, peak shear and maximal flow were not significantly different between H-FMC and non-H-FMC children and adolescents. Peak FMD was significantly lower in H-FMC individuals than in non-H-FMC.

A correlation matrix was constructed using FMD, H-FMC, BMI and BMI percentile values. A greater H-FMC was associated with lower peak FMD ($r=0.455$, $P<0.001$). Peak FMD was positively correlated with BMI ($r=0.243$, $P=0.004$) and BMI percentile ($r=0.234$, $P=0.006$). A greater H-FMC was not significantly correlated with increased BMI ($r=-0.006$, $P=0.949$) or BMI percentile ($r=-0.066$, $P=0.442$). A one-way analysis of variance for degree of H-FMC revealed no significant difference between or within groups based on BMI percentile ($P=0.92$).

DISCUSSION

The aim of the study was to examine the prevalence of early FMC during reactive hyperemia in normal weight, overweight and obese children and adolescents and investigate potential differences of anthropometric and hemodynamic measures in relation to its presence. We found that approximately 67% of the population displayed H-FMC. Those individuals who displayed H-FMC had a lower peak FMD compared to individuals that did not.

Jiang et al. (2011) suggested two explanations for transient initial constriction following cuff release: 1) an FMC factor or 2) a reduction in intra-arterial pressure resulting from the fall in downstream resistance that, in turn, led to increased flow. An argument could be made that non-H-FMC children and adolescents could possibly have a blunted shear-stimulated release of a constrictor agent, such as endothelin-1 (Jiang et al., 2011; Kuchan & Frangos, 1993). If this were true, one would expect a difference in the time to peak between H-FMC and non-H-FMC groups, which there was not. A blunted shear stress release of vasoconstrictors such as endothelin-1 could, theoretically, be due to a higher level of shear stress in non-FMC before cuff release, since high levels of shear stress have been shown to suppress endothelin-1 release in cultured endothelial cells (Kuchan & Frangos, 1993). However, in the present study, there was no significant difference in shear stress or maximal flow between subjects who displayed an H-FMC and those who did not.

Another possible explanation may be that H-FMC children have a higher sensitivity to endothelin-1. Research by Weil et al. (2011) in adults supports the notion that overweight and obesity are associated with enhanced endothelin-1 mediated vasoconstriction. Enhanced constriction could contribute to endothelial vasodilator dysfunction and to the increase risk of hypertension in these subjects. In the present study, 85.7% of H-FMC children and adolescents were categorized as overweight or obese versus 68.8% of the non-H-FMC. Those demonstrating H-FMC to had a nonsignificantly greater BMI percentile, which may support the hypothesis of the release

of FMC factor following cuff release. However, H-FMC and BMI percentile were not significantly correlated.

A significant drop in intra-arterial pressure may also be responsible for H-FMC. Reactive tissue hyperemia is due to the buildup of metabolic by-products during ischemia, inducing vasodilation of "resistance" arteries (at the microcirculation level). The resulting fall in circulatory resistance led to increased blood flow in the "conduit" artery upstream, so that its own resistance to flow may become significant (at least until its own vasodilation occurs) and create a pressure loss, which may cause its passive mechanical constriction (recoil). However, all things being equal, this response should have been observed in all subjects because the methodology was identical for H-FMC and non-H-FMC children and adolescents. Differences in amplitude of tissue hyperemia and/or in baseline conduit artery diameter might explain various degrees of imbalance between these opposite mechanisms, with H-FMC occurring when greater hyperemia and/or smaller conduit artery baseline diameter led to a larger pressure drop. This, in turn, would result in a greater, although delayed, FMD, which we could not observe in the present study. Moreover, we found no significant difference in shear stress or maximal flow between subjects who displayed an H-FMC and those who did not, and children and adolescents with an H-FMC had a significantly lower FMD than those without. Therefore, some release of endothelin-1 cannot be ruled out.

FMD has been utilized as a predictor of cardiovascular events in asymptomatic subjects (Thijssen et al., 2011). Overweight and obese children have displayed impaired FMD values (Meyer et al., 2006) and are at increased risk for developing coronary heart

disease risk factors (Freedman et al., 1999). Higher FMD is generally associated with healthy endothelial function, and in the present study, non-H-FMC children and adolescents displayed brachial artery FMD levels similar to levels of healthy control subjects in other studies (Babar et al., 2011). Even though the presence of an H-FMC was associated with lower FMD response, it is unclear whether the H-FMC is associated with other cardiovascular risk factors. This will need to be examined in future studies.

Of interest, when an addition of the percentage of H-FMC to peak FMD is applied to those displaying a vasoconstriction, the difference in peak FMD between the two groups disappears. It seems that there is a limited timeframe during which peak dilatory response to reactive hyperemia occurs. Therefore, if the initial response to cuff release is constriction, the result will lower the overall dilation of the brachial vessel relative to the initial baseline diameter. Statistical difference has been observed in the current study between the two groups for the composite end point and FMD values (Table 2). H-FMC may have similar complementary value to FMD as L-FMC, which was shown to have further value than a composite value (FMD+L-FMD) by reducing false-positive and false-negative results when used independently of FMD in clinical applications (Humphreys et al., 2014; Gori et al., 2010). Not only does the presence of an H-FMC complicate the analysis of FMD but it also adds to the body of FMD research. The time course for dilation for both groups is similar, which seems to imply that NO and other dilating factors are secreted for a set amount of time in children and adolescents. BMI may also have an effect on the prevalence of vasoconstriction observed after occlusion, with H-FMC being a possible independent indicator of vascular change. Since obesity is

often associated with endothelial dysfunction (Tounian et al., 2001; Williams et al., 2005), the presence of a H-FMC may be an initial sign of vascular dysfunction, as there was a moderate correlation between H-FMC and FMD but with BMI or BMI percentile. However, this hypothesis will need to be examined in future studies.

Previous research has investigated ancillary measures to FMD. L-FMC has been documented as a response of the radial artery during distal occlusion (Gori et al., 2008; Gori et al., 2010). This measure provides an accurate measure of resting arterial tone as it assesses the arterial response to resting shear stress (Gori et al., 2008; Gori et al., 2010). However, the technique reported for measuring L-FMC is vastly different compared to H-FMC. Weissgerber et al. (2010) reported that L-FMC was observed in the radial artery but not in the brachial artery of healthy pregnant and nonpregnant women. It would appear that L-FMC in the brachial artery is not as uniform a response as the radial artery. A significant increase in brachial artery diameter during cuff occlusion was observed in healthy children and young adults (Thijssen et al., 2008). Not only does the imaged vascular bed differ but also the time course of the constriction is different. L-FMC measures constriction during blood flow restriction while H-FMC is the constriction observed immediately following occlusion release. Brachial artery H-FMC may have a comparable benefit on cardiovascular health assessment as L-FMC of the radial artery.

The present study is subject to a few limitations. Intra-arterial pressure and vasoconstrictor mediators were not directly measured. Since many of the participants were recruited from a pediatric weight management clinic (in addition to the community), the percentage of overweight and obese children in this study was much higher than in

the general pediatric population. Future studies could investigate children with lower BMI values for the presence and rate of an H-FMC as well as the prevalence of an H-FMC in differing adult populations. Investigation of health risk factors possibly associated with an H-FMC would also be beneficial. In addition, assessment of L-FMC would be beneficial when assessing FMD and H-FMC in future studies. A prospective study would be required to determine the validity and reliability of H-FMC as a risk factor for atherosclerosis and cardiovascular disease.

CONCLUSION

We observed an H-FMC in a majority of overweight and obese children and adolescents during reactive hyperemia. FMD was lower in children and adolescents who experience this phenomenon, suggesting the presence of endothelial dysfunction. Whether H-FMC has any clinical implications is currently unknown. However, our findings suggest that this phenomenon should be considered when analyzing and interpreting FMD data. Future studies should examine the relationship of the presence of H-FMC with cardiovascular risk factors in children and adults.

TABLE LEGENDS

Table 1. Demographic Characteristics

Table 2. Measures of Vascular Function

Table 1. Demographic Characteristics

	H-FMC	Non-H-FMC	P-value
n (% male)	91 (44%)	45 (47%)	
Age, years	13.0±2.5	12.8±2.7	0.602
Blood pressure, mmHg			
Systolic	118±12	117±13	0.849
Diastolic	61±9	60±8	0.480
Height, cm	158.8±12.1	155.9±14.5	0.227
Weight, kg	76.9±26.4	70.3±26.3	0.171
Tanner Stage	3±1	3±1	0.477
Hip Circumference, cm	100.5±16.3	97.4±17.4	0.308
Waist Circumference, cm	87.2±16.7	83.5±17.8	0.235
Body Mass Index, kg/m^2	29.8±7.5	27.8±7.8	0.151
Body Mass Index Percentile,	91.8±14.9	84.6±22.8	0.057

Note: Values are means±SD; *n*, number of participants/group.

H-FMC – High-Flow Mediated Constriction

Non-FMC – No High-Flow Mediated Constriction

Table 2. Measures of vascular function

	H-FMC	Non-H-FMC	P-value
Brachial Artery Diameter, mm	3.33±0.44	3.24±0.40	0.296
Time to Peak FMD, sec	59.0±24.3	62.1±34.3	0.591
Average Shear, sec^{-1}	262.5±62.1	273.7±61.1	0.321
Peak Shear, sec^{-1}	294.4±74.6	301.5±65.6	0.589
Maximal Flow, m/s	0.85±0.18	0.89±0.17	0.168
Brachial Artery Peak Diameter, mm	3.56±0.47	3.55±0.40	0.826
Flow-Mediated Dilation, %	5.43±3.41	8.05±3.97	<0.001
H-FMC+FMD, %	8.03±3.27	8.05±3.97*	0.977

Note: Values are means±SD; *Non-H-FMC is the same for FMD and H-FMC+FMD

Maximal flow (m/s) is a measure of average blood flow following cuff release.

FMD – Flow Mediated Dilation

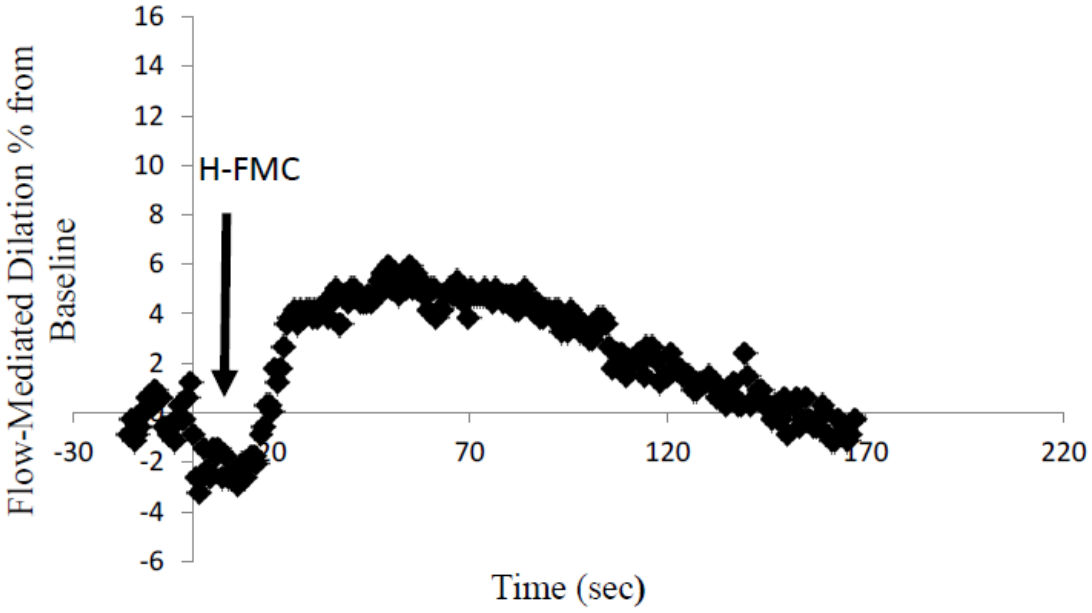
H-FMC – High-Flow Mediated Constriction

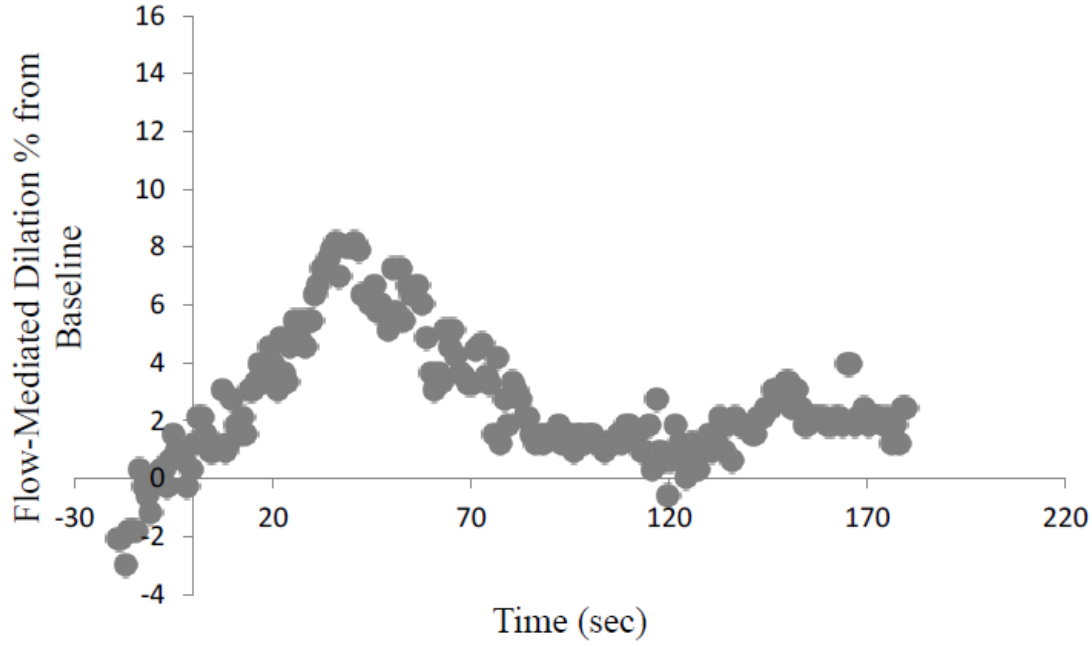
Non-FMC – No High-Flow Mediated Constriction

FIGURE LEGEND

Figure 1. A representation of a Flow-Mediated Dilation (percent change in brachial artery diameter from baseline) response in H-FMC (♦) and Non-H-FMC (●) children.

Figure 1


Flow-Mediated Dilation % from Baseline
H-FMC
16
14
12
10
8
6
4
2
-2
-4
-6
-30
20
70
120
170
220
Time (sec)


Flow-Mediated Dilation % from Baseline
16
14
12
10
8
6
4
2
0
-2
-4
-30
20
70
120
170
220
Time (sec)

CHAPTER 5. HIGH-FLOW MEDIATED CONSTRICTION IN ADULTS IS NOT INFLUENCED BY BIOMARKERS OF CARDIOVASCULAR AND METABOLIC RISK

High-Flow Mediated Constriction is Not Influenced by Biomarkers of Cardiovascular and Metabolic Risk

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Short Title: H-FMC in Adults

Key Words: Ultrasound, Reactive Hyperemia, Flow-Mediated Dilation, Adults

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Summary

Purpose: During reactive hyperemia, the brachial artery in some individuals constricts prior to dilation. The aim of this study was to describe the frequency of high-flow-mediated constriction (H-FMC) in adults, and relationship of H-FMC to body composition and biomarkers of cardiovascular and metabolic risk.

Methods: 246 adults (124 male, 122 female; 36±7 years) were assessed for H-FMC via ultrasound imaging of the brachial artery. Blood pressure, glucose, insulin, lipids, and body composition assessed via dual energy X-ray absorptiometer were collected for each participant. H-FMC was characterized as a 10-second average of maximal post-occlusion constriction. Independent t-tests were used to compare variables between H-FMC vs. Non-H-FMC individuals.

Results: H-FMC was observed in approximately 69% of adult participants (54 obese, 57 overweight, and 59 normal-weight). Total body mass (82.3±17.5vs.76.3±16.3kg, P=0.012), fat mass (27.7±11.5vs.23.8±10.5kg, P=0.012), body mass index [BMI:27.7±4.9vs.26.1±5.0kg/m^2, P=0.018], and low-density lipoprotein to high-density lipoprotein ratio [LDL/HDL:2.41±1.03vs.2.09±0.72, P=0.007] were significantly higher in H-FMC compared to Non-H-FMC individuals. FMD (6.12±3.48vs.8.09±3.02%, P<0.001) was significantly lower in H-FMC subjects. However, when H-FMC was added to FMD there was no significant difference in brachial artery dilation between groups (7.57±3.69vs.8.09±3.02%, P=0.250).

Conclusion: Increased body mass, fat mass and BMI were associated with a greater H-FMC. When H-FMC is present, the FMD response to reactive hyperemia is significantly

decreased. Since H-FMC has been observed to negatively affect FMD response to reactive hyperemia, it is suggested that H-FMC should be noted when analyzing and interpreting FMD data. H-FMC is an indicator of vascular change and may be an ancillary measure of endothelial health.

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide (Mendis et al., 2011). In 2008 over 75% of the 17.3 million CVD deaths were attributed to manifestations of atherosclerosis, an underlying process that narrows and hardens blood vessels (Mendis et al., 2011). Endothelial dysfunction has been shown to be an important precursor in the atherosclerotic process (Ross, 1993). Flow-mediated dilation (FMD) is widely used as a noninvasive method for measurement of endothelial health in children and adults (Corretti et al., 2002).

Previously, we have reported that 67% of children demonstrate vasoconstriction prior to a shear stress-induced nitric oxide (NO)-mediated dilation of the brachial artery during an FMD assessment (Ostrem et al., 2015). The opposing vasoconstrictor stimulus produces a biphasic response when plotting the diameter change of an artery in response to reactive hyperemia (Figure 1). This physiological response has been referred to as a high-flow-mediated constriction (H-FMC) (Jiang et al., 2011; Ostrem et al., 2015). It has been postulated that the observed vasoconstriction could result from a hemodynamic effect of increased blood flow leading to decreased transmural pressure and/or a shear stress-stimulated constrictor release, such as endothelin-1 (Dobrosielski et al., 2006; Jiang et al., 2011; Kuchan & Frangos, 1993; Ostrem et al., 2015; Turner, 2011).

This biphasic response of the brachial artery has previously been reported in adult males during reactive hyperemia (Dobrosielski et al., 2006). To our knowledge, no published work has reported on the frequency and magnitude of H-FMC observed at the brachial artery in a healthy adult male and female population or the impact of an H-FMC

on reported peak FMD. Additionally, the relationship of biomarkers of cardiovascular and metabolic health and H-FMC has not been elucidated. The presence of an H-FMC could possibly negatively affect peak FMD such that the response to reactive hyperemia in healthy individuals appears similar to individuals with endothelial dysfunction and increased cardiovascular risk (Neunteufl et al., 1997). The purpose of this study was to describe the frequency and magnitude of an H-FMC during reactive hyperemia in healthy adult males and females. In addition, we sought to examine the relationship of H-FMC to FMD, biomarkers of cardiovascular and metabolic risk as well as measures of body composition. It is hypothesized that an H-FMC affects peak FMD and is significantly correlated with body composition as well as known cardiovascular and metabolic risk factors.

Materials and Methods

Study Population

The 246 adults (124 males, 122 females) in this study were randomly sampled from two population based longitudinal studies investigating the development of obesity and insulin resistance and their interaction with associated cardiovascular risk factors (Dengel et al., 2011; Marlatt et al., 2013). Subjects who smoked or were taking prescription medications, such as blood pressure, insulin, dyslipidemia or statin medication, were excluded from the study. The study protocol was reviewed and approved by an institutional review board, and all participants gave written informed consent.

Physical Assessments

Measurements for height and weight were obtained with a standard stadiometer (Avrton, Model S100, Prior Lake, MN, USA) and electronic scale (ST Scale-Tronix, Serial No. 5022-8893, White Plains, NY, USA), respectively. Body mass index (BMI) was calculated as weight in kilograms (kg) divided by height in meters-squared (m^2).

Dual-energy X-ray Absorptiometry

Body composition was measured using a dual energy X-ray absorptiometer (DXA) (GE Healthcare Lunar iDXA, platform version 13.6, GE Healthcare Lunar, Madison, Wisconsin, USA). The total body scan was analyzed using the enCore™ CoreScan software. Standard imaging and positioning protocols were implemented while subjects were fasted and hydrated. Proprietary software algorithms from the manufacturer were used to segment and analyze the body into upper and lower extremities using the standard regions of interest (ROI) and then combined for each of the measures of interest: lean mass, fat mass, visceral adipose tissue mass (VAT), android fat, gynoid fat, subcutaneous (subQ) fat, and bone mass. Android fat was calculated between the ribs and the top of the pelvis, while the gynoid fat was determined from the upper thighs to the top of the pelvis. The subQ fat was then calculated as the android fat–VAT mass. Each subject's measure of interest was reported in kilograms (kg).

Glucose, Insulin and Lipid Profile Assessment

All assays were conducted with standard procedures at a Center for Disease Control and Prevention-certified laboratory. Fasting blood samples were collected for glucose, insulin and lipid levels including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), and low-density lipoprotein cholesterol (LDL). The LDL/HDL ratio was reported for each subject, because a higher ratio has been related to increased coronary heart disease risk (Fernandez & Webb, 2008). Homeostatic model assessment (HOMA) of insulin resistance and β-cell function were calculated using fasting plasma insulin and fasting plasma glucose (Matthews et al., 1985).

Vascular Assessments

Vascular assessment was conducted for all participants following at least 8 hours of fasting. Subjects were required to refrain from caffeine ingestion within 4 hours prior to testing and avoid strenuous exercise or physical activity 24 hours prior to the study visit. Subjects were tested in a quiet, climate-controlled room (22-23°C). Resting blood pressure was recorded using an automated sphygmomanometer (Colin BP-8800, Colin Medical Instruments Corp., San Antonio, TX, USA) on the right arm prior to FMD assessment. A specialized occlusion cuff (D.E. Hokanson, Inc., Bellevue, WA) was placed approximately five centimeters (cm) distal to the antecubital space on the left forearm. Following 15 minutes of quiet rest in the supine position, vascular images of the left brachial artery were obtained proximal to the antecubital fossa in the longitudinal plane using a conventional ultrasound scanner (Acuson, Sequoia 512, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with a 8-15 MHz linear array probe held at a constant pressure on the skin and at a fixed point over the imaged artery by a

stereotactic arm. The occlusion cuff on the left forearm was then inflated to a suprasystolic pressure level of 200 mmHg and maintained for 5 minutes. Vascular images were captured 20-seconds prior to cuff release until 3 minutes post-cuff release and were digitized and stored on a personal computer for later analysis using an electronic wall-tracking software program (Vascular Research Tools 5, Medical Imaging Application, LLC, Iowa City, IA, USA).

A trained sonographer performed all digital ultrasound image capturing and analysis. The coefficient of variation was 11.1% in our laboratory when FMD was measured 7 days apart, demonstrating good reproducibility. Vascular images were assessed for brachial artery baseline diameter, maximal blood flow, peak shear and subsequent brachial artery constriction and dilation. The baseline brachial artery measurement was defined as a 10-second average just prior to blood pressure cuff release, i.e. occlusion baseline, to negate any low-flow mediated constriction that may occur during occlusion. Maximal blood flow (m/s) was defined as the largest 10-second average rate of blood flow following 3 seconds post-cuff release. Peak shear was used to estimate the greatest shear stress during the same timeframe as maximal blood flow. Peak shear was calculated as a 10-second average during post occlusive reactive hyperemia, which was calculated as blood flow velocity divided by arterial diameter. H-FMC was observed and characterized using a 10-second average of the lowest point from baseline brachial artery diameter following 3-seconds post cuff release and considered present if the percent change was less than -0.1% (Figure 1). Peak dilation during each procedure was defined as the 10-second average of the greatest percent change from baseline

brachial artery diameter. The time that elapsed between cuff release and greatest constriction in H-FMC individuals was recorded as time-to-trough FMC (TTT FMC). There was no observed constriction in Non-H-FMC individuals following cuff release, so TTT FMC was not applicable for the Non-H-FMC group. The time between cuff release and peak dilation was recorded as time-to-peak FMD (TTP FMD).

Statistical Analysis

All statistical analysis was performed using IBM SPSS Statistics 22 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Descriptive statistics were conducted for the two groups on anthropometric, biomarkers of cardiovascular and metabolic risk, and FMD measurements. Allometric scaling was performed for H-FMC and FMD related to baseline brachial artery diameter as proposed by Atkinson and colleagues (Atkinson & Batterham, 2013; Atkinson et al., 2013). Independent t-tests were used to compare variables between subjects who demonstrated an H-FMC vs. Non-H-FMC. An alpha value of 0.05 was denoted as statistically significant. A correlation matrix was then constructed which included significant body composition, biomarkers of cardiovascular and metabolic risk, and FMD measures that were differing between H-FMC and Non-H-FMC groups at a significance of $P<0.05$. A subgroup analysis of significant variables between groups was conducted within genders.

Results

Mean demographic characteristics of adults are presented in Table 1. Adults ranged in age from 18 to 46 years old. Of the 246 adults examined in this study, 170 (69.1%) displayed an H-FMC. A greater magnitude of H-FMC was positively associated with lower peak FMD ($r=0.238$, $P<0.001$). No differences were noted between the two groups for gender composition ($P=0.114$), however, 73% of males and 65% of females displayed an H-FMC. Height, VAT mass and body fat percentage were not different between groups. Body mass index (BMI) of the study population ranged from 17.2 to 39.3, with 79.4% of obese individuals, 65.5% of overweight individuals and 64.8% of normal-weight individuals displayed an H-FMC. Total body mass, BMI, fat mass, subQ fat, android fat, gynoid fat and bone mass were significantly higher in H-FMC adults compared to Non-H-FMC adults. Total body mass ($r=-0.127$, $P=0.047$) and BMI ($r=-0.140$, $P=0.028$) were negatively associated with H-FMC. Fat mass ($r=-0.119$, $P=0.063$), subQ fat ($r=-0.123$, $P=0.059$), android fat ($r=-0.091$, $P=0.164$) and gynoid fat ($r=-0.111$, $P=0.089$) were not significantly associated with H-FMC.

A subgroup analysis within genders was conducted on significantly correlated variables between H-FMC and Non-H-FMC groups. There was not a significant correlation within males for total body mass ($r=-0.039$, $P=0.669$), BMI ($r=-0.059$, $P=0.515$), fat mass ($r=-0.023$, $P=0.802$), or subQ fat ($r=-0.053$, $P=0.567$) with H-FMC. However, significant correlations were found for total body mass ($r=-0.205$, $P=0.024$), BMI ($r=-0.205$, $P=0.024$), fat mass ($r=-0.217$, $P=0.016$), and subQ ($r=-0.202$, $P=0.030$) with H-FMC in females.

The comparison of the blood pressure and blood profile between H-FMC and Non-HFMC groups is represented in Table 2. There was no significant difference between groups for systolic and diastolic blood pressure, glucose, insulin, HOMA, total cholesterol, triglycerides, HDL or LDL. However, LDL/HDL was significantly elevated in H-FMC subjects compared to Non-H-FMC subjects (Table 2), but not significantly correlated to H-FMC ($r=-0.111$, $P=0.087$).

Table 3 displays the study population's vascular measures. Baseline brachial artery diameter in H-FMC individuals was not significantly different from Non-H-FMC individuals. In H-FMC individuals, the average TTT FMC was 10.2±4.9 seconds. Peak shear, maximal flow and TTP FMD were not significantly different between H-FMC and Non-H-FMC adults. Peak FMD response was significantly greater in Non-H-FMC individuals and did not change when using allometric scaling. However, when the magnitude of H-FMC was added to FMD in the H-FMC group, there was no longer a significant difference is response to reactive hyperemia (7.57±3.69 vs. 8.09±3.02, $P=0.250$). Peak FMD was negatively correlated with total body mass ($r=-0.197$, $P=0.002$), and bone mass ($r=-0.279$, $P<0.001$). Gynoid fat ($r=0.129$, $P=0.048$) was positively associated with peak FMD.

Discussion

The purpose of the present study was to examine the prevalence of H-FMC during reactive hyperemia in healthy adults and to investigate potential differences of body composition, glucose, insulin, HOMA, lipids and FMD measures relative to H-FMC

presence and magnitude. We observed that approximately 69% of the study population displayed H-FMC. Individuals that displayed an H-FMC had a lower peak FMD from an occlusion baseline compared to individuals not displaying an H-FMC. Also, H-FMC individuals had a significantly greater total body mass, BMI, fat mass, bone mass, android fat, gynoid fat, subQ fat and LDL/HDL compared to Non-H-FMC individuals.

Previously we demonstrated that H-FMC prevalence in children and adolescents was approximately 67% (Ostrem et al., 2015), which is very similar to the prevalence observed in adults in the present study. The significant presence of an initial constrictive response to reactive hyperemia in these studies should alert researchers utilizing FMD methodology to the possibility of a blunted peak FMD when using a baseline artery diameter measured before occlusion cuff release. However, when an addition of the percent of H-FMC to peak FMD is applied, the difference in peak FMD between the two groups is negligible. This finding is consistent with our previous research in children and adolescents (Ostrem et al., 2015). Addition of H-FMC may be a better measure of true NO-mediated dilation, since dilation would be assessed from the lowest artery diameter. Thus, researchers could use a baseline diameter measure that occurs after the occlusion cuff has been released. It would appear that an appropriate timeframe for calculation of a 10-second average baseline diameter following occlusion would be between 5 to 15 seconds, since the average TTT FMC was at 10 seconds. This is in contrast to previous research showing that using a universal baseline diameter calculated following occlusion cuff did not produce practical differences in FMD (Ostrem et al., 2015). Flow-mediated dilation has been utilized as a measure of endothelial function and predictor of

atherosclerotic development (Halcox et al., 2009) and cardiovascular events in asymptomatic subjects (Thijssen et al., 2011). A larger FMD response is generally associated with healthy endothelial function. In the current study, Non-H-FMC adults displayed brachial artery FMD levels similar to levels of healthy control subjects in other studies (Bots et al., 2005; Williams et al., 2005) while H-FMC individuals had a significantly lower FMD response. Even though the increased magnitude of an H-FMC was mildly associated with lower FMD response, it is unclear whether the H-FMC is an independent risk factor of endothelial dysfunction.

Age, blood pressure, peak shear, maximal flow and baseline brachial artery diameter were not significantly different between H-FMC and Non-H-FMC groups, also consistent with prior work in children (Ostrem et al., 2015). However, contrary to the findings in children and adolescents, total body mass and BMI were significantly higher in the adult H-FMC group. Since body composition was assessed by DXA in the present study and not in our previous research, more precise weight-related factors were found to be significantly higher in the adult H-FMC group: fat mass, android fat, gynoid fat, subQ fat and bone mass. These data would suggest that body composition has a greater influence on H-FMC in adults compared to other cardiovascular and metabolic risk factors since the LDL/HDL ratio was the only significant cardiovascular or metabolic factor significantly different between groups. However, the correlation of total body mass and BMI with H-FMC are relatively weak. Previous research has demonstrated reduced FMD in abdominally obese individuals compared to lean individuals, suggesting impaired endothelial function associated with android fat (Davison et al., 2010; Williams

et al., 2005). Furthermore, modest increases in visceral fat, as opposed to subQ fat, have resulted in impaired endothelial function (Romero-Corral et al., 2010). However, android fat, gynoid fat and subQ fat were significantly elevated in the H-FMC group in the current study. Historically, gynoid and subQ fat have been considered to be lower health risks compared to VAT and android fat (Samsell et al., 2014). Lower extremity adiposity, specifically larger subQ thigh fat, has even been associated with lowering potential health risks (Snijder et al., 2005). A positive correlation was found between gynoid fat and FMD in the present study, which is consistent with the notion of gynoid fat lowering potential risk. An increased bone mass was also observed in the H-FMC group than the Non-H-FMC group. Since a greater percentage of obese subjects were in the H-FMC group, it would seem appropriate for there to be a significant difference in bone mass between groups due to the well-established positive effect that increased mechanical loading, via body weight, has on bone formation (Cao, 2011). Overweight and obesity has been associated with enhanced endothelin-1 mediated vasoconstriction (Weil et al., 2011) and a large percentage of obese individuals in the current study displayed an H-FMC. It may be possible that an H-FMC in some adults occurs due to increased presence of a flow-mediated constriction factor following cuff release. Since H-FMC was significantly correlated to an increased BMI and body mass, it could be possible that an enhanced endothelin-1 mediated response is partially involved in the H-FMC. However, endothelin-1 was not measured and both BMI and body mass have a relatively weak correlation with H-FMC.

Another point of interest in the current research is the lack of significant differences between groups for many known cardiovascular risk factors. Previous research has reported that many of the known cardiovascular risk factors, such as dyslipidemia (Sorensen et al., 1994), diabetes mellitus (Clarkson et al., 1996), hypertension (Benjamin et al., 2004) and central obesity (waist circumference and VAT) (Hashimoto et al., 1998; Parikh et al., 2009; Romero-Corral et al., 2010) can alter FMD. Of the known cardiovascular risk factors, the LDL/HDL ratio was the only non-weight-related factor statistically significant between the H-FMC and Non-H-FMC groups. However, a larger LDL/HDL ratio has been reported to have a significant associated with increasing BMI (Wilsgaard & Arnesen, 2004). Therefore, it may be possible for the development of abdominal obesity to precede a decline in lipids or presence of other cardiovascular risk factors.

In the current study, there was homogeneity of the study participants for many of the cardiovascular risk factors due to their healthy control status in the overarching study. This homogeneity of participants could be interpreted as a limitation. On the contrary, an H-FMC may be an independent factor for cardiovascular risk, because homogeneity of known cardiovascular risk did not hinder the observation of H-FMC and Non-H-FMC responses to reactive hyperemia. Instead, there were significant correlations between H-FMC, total body mass and BMI with many other weight-related factors trending toward significance. Previous research has suggested that central obesity appears to precede the appearance of hypertension, glucose abnormalities, and dyslipidemia (Cameron et al., 2008). Thus, H-FMC could possibly be either a complimentary health assessment tool to

FMD or an insignificant artifact. A greater understanding of H-FMC could be achieved with future longitudinal research that included H-FMC magnitude with FMD methodology.

Of interest, when a subgroup analysis based on gender was conducted, all of the H-FMC correlations with body composition were not significant in males, but were significant in females. This could be due to hormonal differences expected between genders. However, the significant correlations found in females between body composition and H-FMC remained weak.

It has been posed that FMD, and subsequently H-FMC, needs to be scaled to account for the reliance on baseline diameter in FMD calculation (Atkinson & Batterham, 2013; Atkinson et al., 2013). However, when allometric scaling was performed in the current study, a change in significance between groups for FMD was not observed (Table 3). Furthermore, allometric scaling of H-FMC did not adhere to the criteria outlined in previous research (Atkinson & Batterham, 2013; Atkinson et al., 2013).

Historically, the benefit of FMD testing is the simplistic and noninvasive nature of the procedure. Taking into account the presence of an H-FMC does complicate the analysis of FMD, as does allometric scaling (Atkinson & Batterham, 2013; Atkinson et al., 2013), but it also adds to the body of FMD research. The time to reach peak dilation for both groups is similar, which seems to imply that endothelial NO and other dilating factors are secreted for a set amount of time in adults. Since body mass and BMI seem to have a significant, but weak, correlation with the prevalence of vasoconstriction observed post-occlusion, H-FMC may be a possible independent indicator of vascular remodeling.

Obesity could be an extrinsic factor associated with endothelial dysfunction, while an H-FMC may be an intrinsic sign of vascular dysfunction. However, this hypothesis will need to be examined in future studies.

The present study is subject to a few limitations. Intra-arterial pressure and vasoconstrictor mediators were not directly measured. The possibility of autonomic nervous system contribution to H-FMC was also not investigated. The cross-sectional nature of the study also does not answer the reproducibility of the H-FMC response to blood flow manipulation. Future studies could investigate both children and adult cohorts for the reproducibility in presence and magnitude of an H-FMC. Also, at-risk and diseased populations could be investigated for prevalence of an H-FMC. The investigation of inflammatory and other ancillary blood markers associated with an H-FMC would also be beneficial. A prospective study would be required to determine the reproducibility, validity, and physiological modification of an H-FMC as a risk factor for atherosclerosis and cardiovascular disease.

Conclusion

An H-FMC occurs in a majority of healthy adults during reactive hyperemia and FMD was lower in adults who experience H-FMC, suggesting the presence of endothelial dysfunction. Since a greater percentage of children, adolescents (Ostrem et al., 2015) and adults seem to display an H-FMC following occlusion, a post-occlusion baseline may be worth consideration in future FMD technique. Increased body mass and BMI were associated with a greater H-FMC; however, it is currently unknown whether H-FMC has

any clinical implications. Nevertheless, our findings suggest that this H-FMC should be considered when analyzing and interpreting FMD data. Future studies should examine the reproducibility, validity, and reliability of H-FMC and changes in body composition, specifically body mass and fat mass, on H-FMC magnitude in children and adults.

TABLE LEGENDS

Table 1. Physical Characteristics

Table 2. Blood Profile

Table 3. Measures of Vascular Function

Table 1. Physical Characteristics

	H-FMC	Non-H-FMC	P-value
n (% male)	170 (54%)	76 (43%)	
Age, years	36.3±7.0	36.8±6.6	0.597
Height, cm	172.0±11.7	170.9±11.7	0.483
Total body mass, kg	82.3±17.5	76.3±16.3	0.012
Body Mass Index, kg/m^2	27.7±4.9	26.1±5.0	0.018
Bone mass, kg	3.2±0.7	3.0±0.7	0.026
Lean body mass, kg	51.4±11.4	48.7±11.5	0.086
Fat mass, kg	27.7±11.5	23.8±10.5	0.012
Body fat, %	34.2±10.3	32.1±10.6	0.145
VAT mass, kg	0.89±0.71	0.74±0.68	0.122
Android fat, kg	2.5±1.3	2.1±1.2	0.029
Gynoid fat, kg	4.6±1.7	4.1±1.6	0.037
SubQ, kg	1.6±0.8	1.4±0.8	0.023

Note: Values are means±SD; *n*, number of participants/group; DXA, dual energy x-ray absorptiometry; H-FMC – High-Flow Mediated Constriction

Non-FMC – No High-Flow Mediated Constriction

Table 2. Blood Profile

	H-FMC	Non-H-FMC	P-value
SBP, mmHg	113±13	113±13	0.845
DBP, mmHg	70±10	70±11	0.698
Glucose, $mg \cdot dL^{-1}$	101±13	99±11	0.205
Insulin, mU/l	7.7±7.1	6.4±5.7	0.148
HOMA1-IR	35.9±36.0	28.7±26.9	0.125
HOMA1-%B	1.5±1.3	1.3±1.1	0.201
Triglycerides, $mg \cdot dL^{-1}$	130±103	117±103	0.407
Total Cholesterol, $mg \cdot dL^{-1}$	183±36	178±39	0.306
HDL, $mg \cdot dL^{-1}$	49±14	51±12	0.235
LDL, $mg \cdot dL^{-1}$	109±32	103±29	0.125
LDL/HDL	2.41±1.03	2.09±0.72	0.007

Note: SBP, systolic blood pressure; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein;

H-FMC – High-Flow Mediated Constriction

Non-FMC – No High-Flow Mediated Constriction

Table 3. Measures of vascular function

	H-FMC	Non-H-FMC	P-value
Brachial Artery Diameter, mm	3.80±0.74	3.65±0.64	0.095
TTP FMD, sec	56.1±25.6	52.0±18.7	0.215
Peak Shear, sec^{-1}	267.0±120.5	255.1±97.1	0.450
Maximal Flow, m/s	0.98±0.39	0.92±0.28	0.257
Flow-Mediated Constriction, %	-1.45±1.18	1.40±1.30	<0.001
Flow-Mediated Dilation, %	6.12±3.48	8.09±3.02	<0.001
Scaled* Flow-Mediated Dilation, %	6.56±3.73	8.67±3.23	<0.001

Note: Values are means±SD;

Maximal flow (m/s) is a measure of average blood flow following cuff release.

TTP FMD - Time-to-Peak FMD from cuff release to peak dilation

FMD – Flow Mediated Dilation

H-FMC – High-Flow Mediated Constriction

Non-FMC – No High-Flow Mediated Constriction

*Scaled Flow-Mediated Dilation, % refers to Allometric scaling[14,15]

Figure Legend

Figure 1. A comparison of a Flow-Mediated Dilation (percent change in brachial artery diameter from baseline) response in H-FMC (♦) and Non-H-FMC (♦) adults.

Figure 2. A scatter plot of the relationship between BMI and H-FMC in adults.

Figure 1.

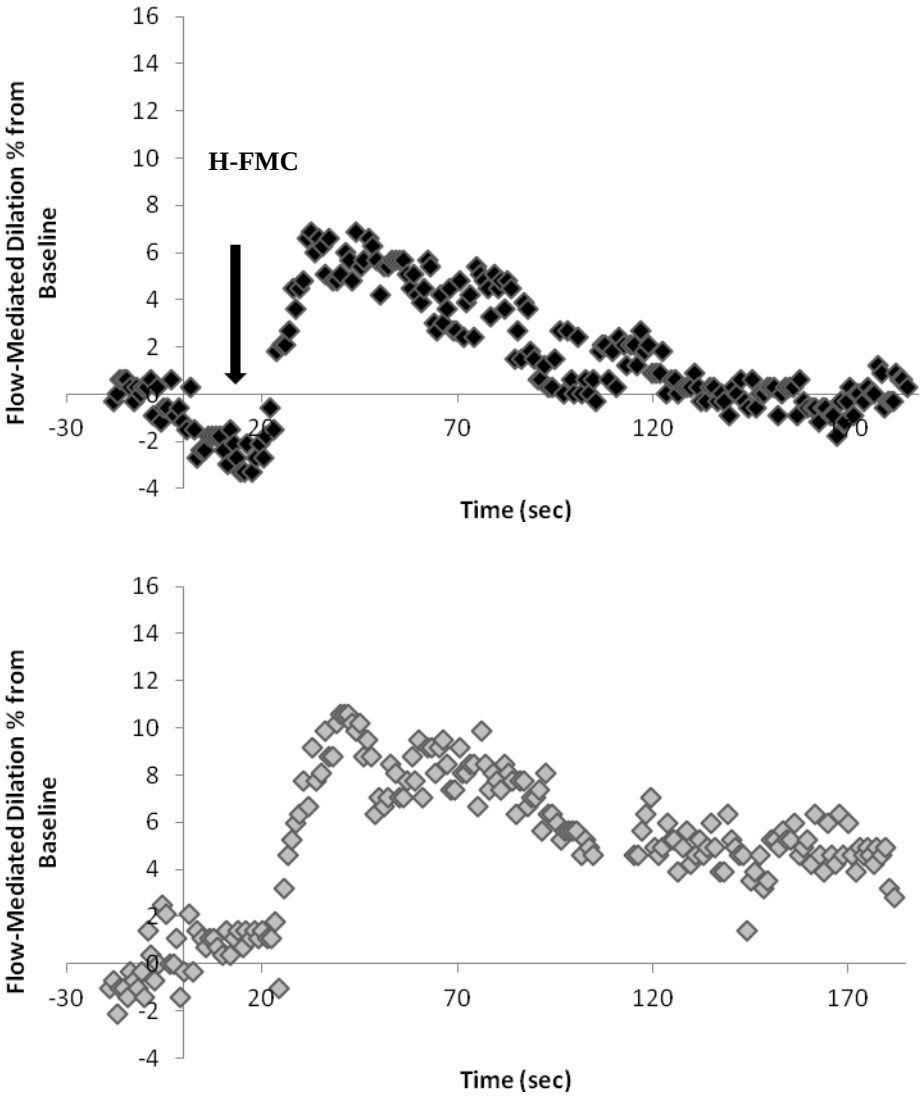
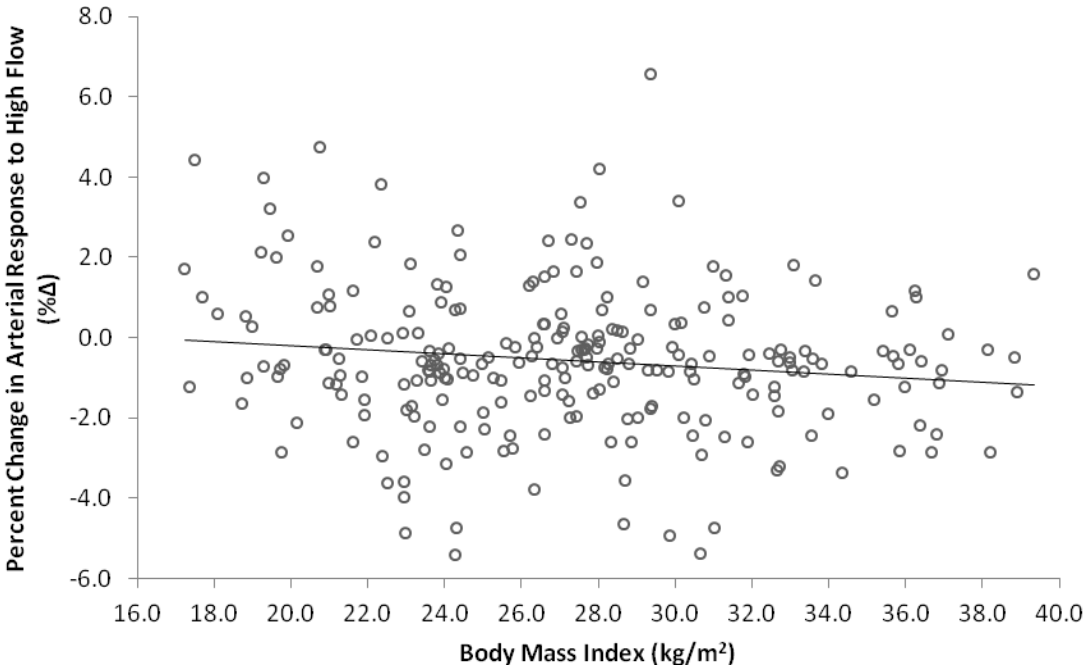

H-FMC
Flow-Mediated Dilation % from Baseline
Time (sec)
16
14
12
10
8
6
4
2
0
-2
-4
-30
20
70
120
170

Figure 2.


Percent Change in Arterial Response to High Flow (%Δ)
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
-6.0
16.0
18.0
20.0
22.0
24.0
26.0
28.0
30.0
32.0
34.0
36.0
38.0
40.0
Body Mass Index (kg/m²)

CHAPTER 6. CONCLUSION

Research Results and Implications

Non-invasive assessment of endothelial function via FMD has been extensively studied since the technique's inception. While brachial artery FMD has been shown to correlate with coronary artery endothelial function and has a positive correlation with endothelial health, studies concerning the H-FMC response during an FMD procedure are lacking. The present dissertation offers insight into understanding H-FMC by quantifying the H-FMC response in various populations during an FMD procedure in three separate studies with one common theme. The main findings of the three studies presented in this dissertation demonstrate the need for consideration of H-FMC during FMD analysis and the ease of implementing H-FMC analysis to FMD, as well as extending previous knowledge of H-FMC reproducibility in young adults and H-FMC presence in children, adolescents and adults.

First, the intra- and inter-day reproducibility of H-FMC in young adults was assessed. Results indicated that H-FMC presence and diameter were highly reproducible within and between visits. The findings support consideration of an extended time period of analysis during FMD technique, one that includes the response of the artery immediately following occlusion, specifically between 5-15 seconds post occlusion. Also, the use of an H-FMC diameter during the 5-15 seconds post occlusion as a baseline diameter should be considered.

Second, H-FMC presence was assessed in normal weight, overweight and obese children and adolescents. Results indicated that a majority of overweight and obese children and adolescents experience H-FMC during reactive hyperemia. Interestingly,

FMD was significantly lower in those that experienced H-FMC compared to children and adolescents that did not when calculating FMD from an occlusion baseline. However, when H-FMC was added to FMD in the H-FMC children and adolescents, no difference between groups was observed in overall dilation. While the exact mechanisms for H-FMC are not clear, a greater amount of H-FMC could either support the presence of endothelial dysfunction or could provide information on vessel distensibility, as it is unclear whether H-FMC is beneficial or negative. The findings suggest that H-FMC should be considered when analyzing and interpreting FMD data in children and adolescents.

And finally, H-FMC presence and correlation to body composition, cardiovascular and metabolic measures was assessed in an adult population in the third and final study. The results of this study indicate similar vessel responses to reactive hyperemia as children and adolescents, with a majority of adults displaying H-FMC. Only increased body mass and BMI were associated with a greater H-FMC. It is unknown whether H-FMC has any clinical implications, whether a sign of endothelial dysfunction or increased distensibility. Nonetheless, the findings suggest that H-FMC should be considered when analyzing and interpreting FMD information.

This dissertation establishes an argument that FMD analysis could be enhanced with the inclusion of H-FMC information. A majority of children, adolescents and adults display H-FMC and H-FMC was found to have acceptable intra- and inter-day reproducibility.

Future Research

While this dissertation demonstrates a large presence of H-FMC in children, adolescents and adults, future studies should include H-FMC information to FMD assessments to confirm these findings. Presently, it is unknown whether H-FMC has any clinical implications or if constriction diameter and H-FMC are relevant pieces of information in diseased populations. Specifically, longitudinal studies should examine the relationship of H-FMC and constriction diameter with cardiovascular risk factors and CVD development. Additionally, future studies in H-FMC should investigate the effect of treatments on H-FMC and constriction diameter in healthy and diseased populations.

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